Agilent 2100 Bioanalyzer
2100 Expert User's Guide

Agilent Technologies

Notices

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Manual Part number: G2946-90004

Edition: May 2005

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About this Manual

Welcome to the User's Guide for the Agilent 2100 expert software. This manual provides beginners and advanced users with information needed to successfully run electrophoretic and flow cytometric assays with the bioanalyzer.

The 2100 expert software allows the control of the bioanalyzer (including diagnostic functions) and, in combination with a LabChip kit, the acquisition, interpretation and result presentation of data generated during the analysis of DNA, RNA, proteins, and cells.

In this Manual

This manual provides bioanalyzer users with the following information:

- "About this Manual" on page 5 gives an overview of the subjects in this manual, and lists major innovations and improvements of the 2100 expert software. It also lists supplemental literature and shows you how to make efficient use of this manual.
- "Quick Start" on page 14 is meant for experienced users. It briefly summarizes the necessary steps to prepare and run an assay.
- "Looking at 2100 Expert" on page 28 shows how to get started with the 2100 expert software, and outlines its main operational possibilities.
- "Running and Evaluating Electrophoretic Assays" on page 45 explains how electrophoretic measurements are made using the bioanalyzer, gives detailed descriptions of all steps necessary to run electrophoretic assays, and shows how to analyze and evaluate results using electropherograms and gel-like images.
- "Running and Evaluating Flow Cytometric Assays" on page 166 explains how flow cytometric measurements are made using the bioanalyzer, gives detailed descriptions of all steps necessary to run flow cytometric assays, and shows how to analyze and evaluate results using histograms and dot plots.
- "Working with Chip Data and Assays" on page 258 shows you what to do to open, save, import and export files, and how to print the results.
- "Administering System Functions" on page 299 is your guideline for configuring the 2100 expert software.
- "Running Instrument Diagnostics" on page 316 shows how to use the diagnostic tests to check the bioanalyzer hardware for proper functioning.

- "Performing Verifications" on page 325 describes how you can validate your bioanalyzer system.
- "Products, Spare Parts, and Accessories" on page 334 lists all parts and accessories—including reorder numbers—that are required for electrophoretic and flow cytometric measurements.
- "Glossary" on page 338 explains terms in context with flow cytometry, electrophoresis, and terms specific to the bioanalyzer software and hardware.

If you have any questions this manual cannot answer, please refer to the supplemental literature listed in "Related Documents" on page 8. If you still have questions, contact Agilent for additional support at:

http://www.agilent.com/chem/labonachip

Related Documents

A collection of supplemental literature is given in the following.

Bioanalyzer Manuals

Publication Number	Title
G2938-90006	Agilent 2100 Bioanalyzer Installation and Safety Manual
G2946-90003	Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide

CD-ROM

Publication Number	Title
G2946-60002	Agilent 2100 Bioanalyzer – How to Use Multimedia CD-ROM

Reagent Kit Guides

The *Reagent Kit Guides* give you information on how to perform specific assays, including sample and chip preparation.

Publication Number	Title
G2938-90300	Kit Guide Binder English (including all *Reagent Kit Guides*)
G2938-90010	Reagent Kit Guide DNA 500 and DNA 1000 Assay
G2938-90020	Reagent Kit Guide DNA 7500 and DNA 12000 Assay

Publication Number	Title
G2938-90030	Reagent Kit Guide RNA 6000 Nano Assay
G2938-90040	Reagent Kit Guide RNA 6000 Pico Assay
G2938-90050	Reagent Kit Guide Protein 200 Plus Assay
G2938-90060	Reagent Kit Guide Protein 50 Assay
G2938-90070	Reagent Kit Guide Cell Fluorescence Assays
G2938-90080	Reagent Kit Guide Cell Fluorescence Checkout Kit

Application Notes and Technical Notes

Application Notes and *Technical Notes* are available from the *Agilent 2100 Bioanalyzer Help Desk* or from the lab-on-a-chip web pages:

http://www.agilent.com/chem/labonachip

Newly Published Documentation

Follow this link to see if there is any new documentation:

http://www.chem.agilent.com/scripts/Library.asp

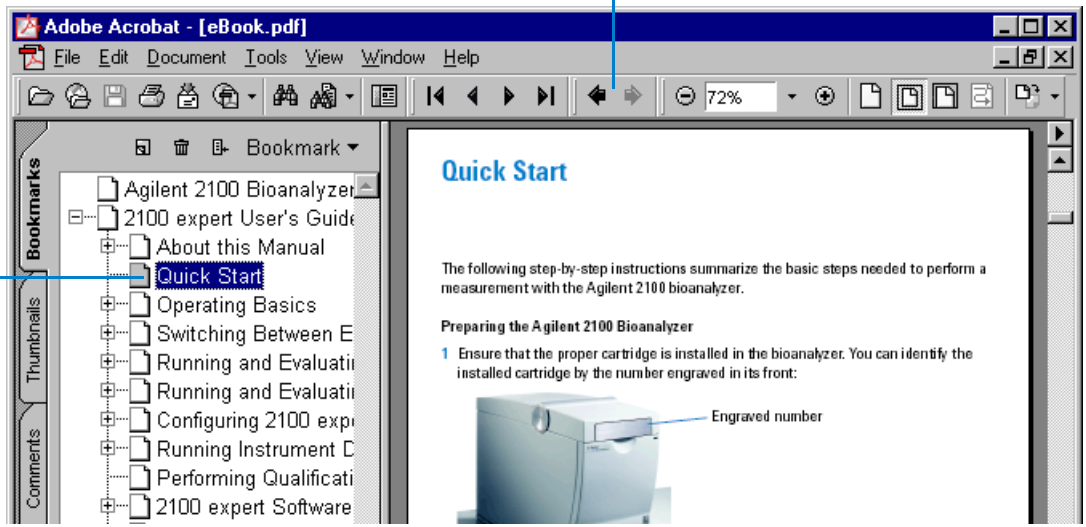
How to Use this Manual

This manual uses convenient online navigation features and follows certain typographic conventions.

Online Navigation

Use the interactive bookmarks in this frame to move to your desired topic.

Use Acrobat Reader's navigation bar to move around within a topic.


Adobe Acrobat - [eBook.pdf]
File Edit Document Tools View Window Help
72%
Bookmark
Bookmarks
Thumbnails
Comments
Agilent 2100 Bioanalyzer
2100 expert User's Guide
About this Manual
Quick Start
Operating Basics
Switching Between E
Running and Evaluati
Running and Evaluati
Configuring 2100 exp
Running Instrument D
Performing Qualificati
2100 expert Software
Quick Start
The following step-by-step instructions summarize the basic steps needed to perform a measurement with the Agilent 2100 bioanalyzer.
Preparing the Agilent 2100 Bioanalyzer
1 Ensure that the proper cartridge is installed in the bioanalyzer. You can identify the installed cartridge by the number engraved in its front:
Engraved number

Click here to go to the table of contents.

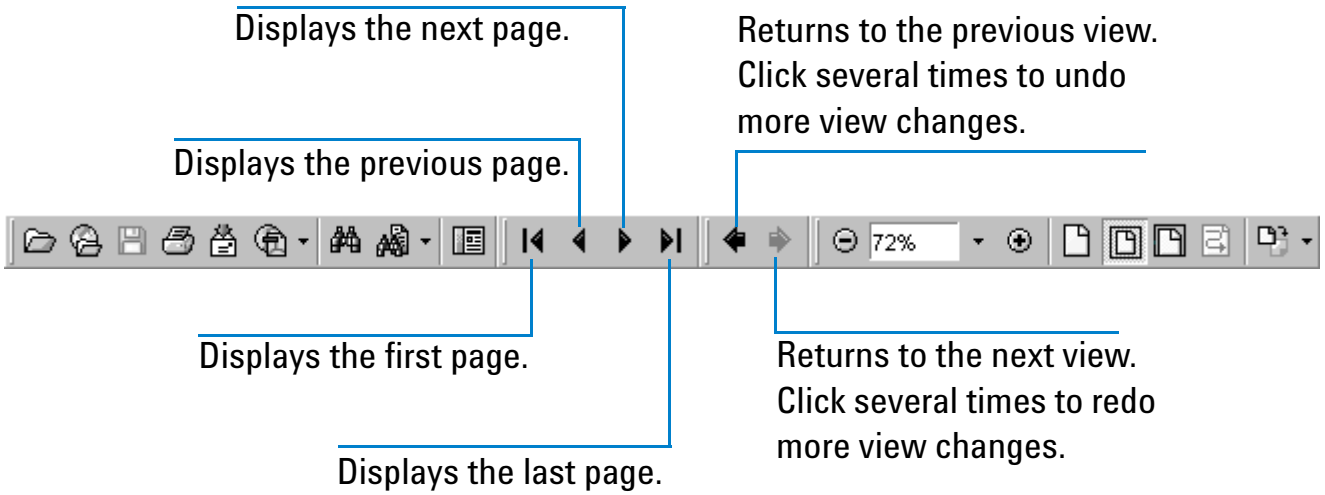
Click here to go to the index.

Here is the current page number.

▲ Displays the previous page.

▼ Displays the next page.

After you have chosen a topic with the bookmarks, use the buttons in Acrobat Reader's toolbar to move around within the topic.


Displays the next page.
Returns to the previous view. Click several times to undo more view changes.
Displays the previous page.
72%
Displays the first page.
Returns to the next view. Click several times to redo more view changes.
Displays the last page.

Layout Conventions

The following typographic conventions are used in this manual:

Highlight	Meaning
Italic	On-screen element *Example*: ... the *OK* button. Emphasis Example: *Right*-click the ... Term Example: *Dot plots* show events as dots. Reference to another document Example: Refer to the *Agilent 2100 Bioanalyzer Troubleshooting and Maintenance Guide*.
Blue	Cross-reference or hyperlink Examples: "Introduction to the Key Features of the 2100 expert" on page 29 http://www.agilent.com/chem/labonachip
`Courier`	Code Example: .. the command line parameter `-port 2` ...
`Courier bold`	User input Example: Enter **`50 MB`**.

Safety Notices, Notes and Tips

Safety notices, notes and tips in this document have the following meaning:

WARNING

A warning notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a warning notice until the indicated conditions are fully understood and met.

CAUTION

A caution notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a caution notice until the indicated conditions are fully understood and met.

NOTE

A note contains important, helpful, or additional information.

TIP

A tip usually points out a timesaving feature.

Quick Start

The following step-by-step instructions guide you through a measurement with the Agilent 2100 bioanalyzer.

Preparing the Agilent 2100 Bioanalyzer

1 Ensure that the proper cartridge is installed in the bioanalyzer. You can identify the installed cartridge by the number engraved at the front.

Engraved number

Note that there are also electrode cartridges without an engraved number.

① = Electrode Cartridge for *electrophoretic* assays
② = Pressure Cartridge for *flow cytometric* assays

2 If you have to change the cartridge, follow the instructions in "Loading the Electrophoresis Chip into the Bioanalyzer" on page 58 or "Loading the Cell Chip into the Bioanalyzer" on page 189, respectively.

Switching on the Agilent 2100 Bioanalyzer

1 Make sure the bioanalyzer is connected to line power and connected to the PC.

2 Turn on the line switch at the rear of the instrument.

The status LED at the front of the bioanalyzer should light up.

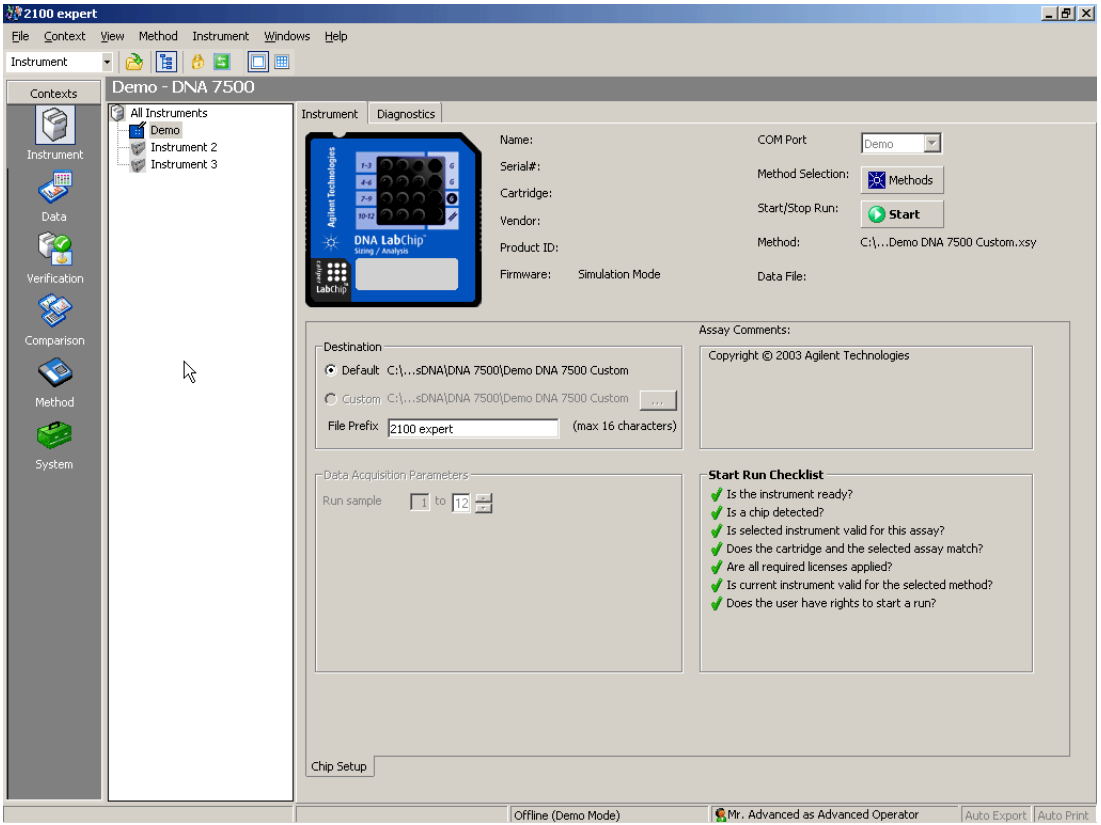
The status LED shows you the current status of the instrument.

Signal	Meaning
Green light	Instrument is switched on and ready for measurement.
Green blinking	Measurement is running.
Orange blinking	Instrument is busy (running self diagnostic, for example).
Red light	Instrument is not ready for measurement. Switch the instrument off and on again. If the problem persists, call Agilent service.

Running a Measurement

1 To start the 2100 expert software on the connected PC, go to your desktop and double-click the following icon:

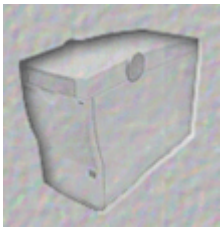
After startup of the software, you enter the *Instrument* context:



NOTE

If you started 2100 expert for the first time after installation, you first need to activate the different software modules with your license keys. See Figure ,"How to Activate Software Licenses" for details.

The *Instrument* tab shows you the status of the bioanalyzer:

Icons	Meaning
	Bioanalyzer detected, lid is open.
	Bioanalyzer detected. Lid is closed, but no chip is inserted.
	No bioanalyzer has been detected. Check the *COM Port* setting (see figure under step 3), the RS 232 connection cable, the power cable, and the power switch. For details on how to set up the bioanalyzer and connect it to a PC, see *Agilent 2100 Bioanalyzer Installation and Safety Guide*.

2 Make sure that a bioanalyzer has been detected before continuing.

3 Select an assay for the chip run.

On the *Instrument* tab, click the *Assays* button.

– OR –

Click the *Assays* menu.

Both will open a menu, allowing you to select an assay for the measurement.

Note that you can also select *File* > *Open File to Run*. This opens a dialog box allowing you to load either an assay (.xsy) or a chip data file (.xad).

4 Prepare the samples and the chip.

For detailed information on sample and chip preparation refer to:

– *Reagent Kit Guides* that are available for each reagent kit

– *Application Notes* that are available for specific kits and applications

---

NOTE

When preparing chip and samples, pay attention to the essential measurement practices described in "Essential Measurement Practices (Electrophoretic Assays)" on page 55 and "Essential Measurement Practices (Flow Cytometric Assays)" on page 187 or as described in the respective Reagent Kit Guide.

---

5 Insert the chip in the Agilent 2100 bioanalyzer:

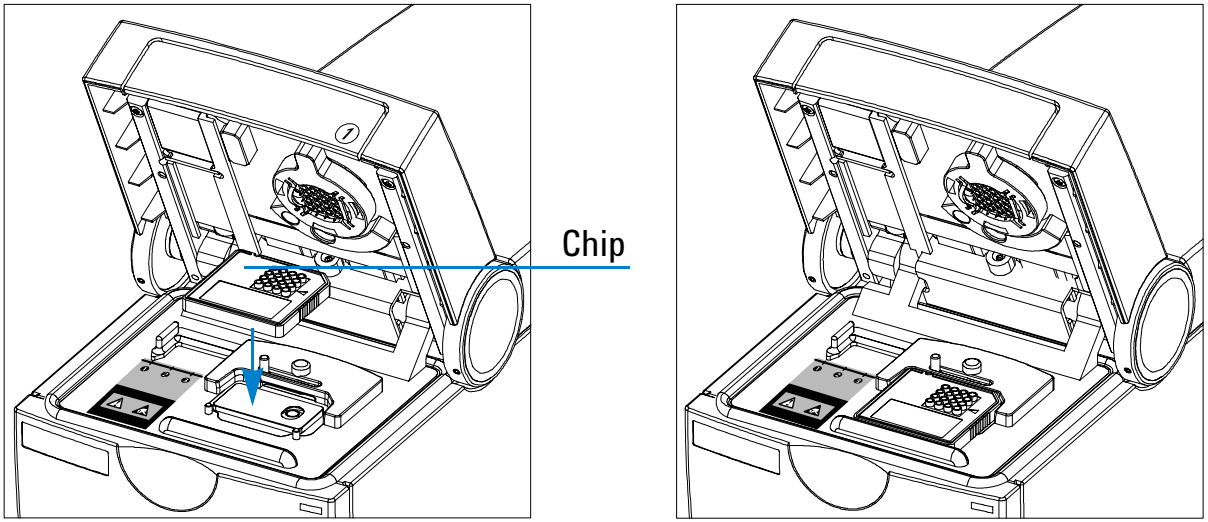
a Open the lid.

The status of the bioanalyzer is updated on the *Instrument* tab.

b Check that the cartridge is inserted properly and the chip selector is in the correct position ("1" for electrophoretic assays, "2" for flow cytometric assays).

For details, please refer to "Loading the Electrophoresis Chip into the Bioanalyzer" on page 58 or "Loading the Cell Chip into the Bioanalyzer" on page 189, respectively.

c Place the chip into the receptacle. The figure shows this for an electrophoresis chip.



The chip fits only one way. Do not force it into place.

CAUTION

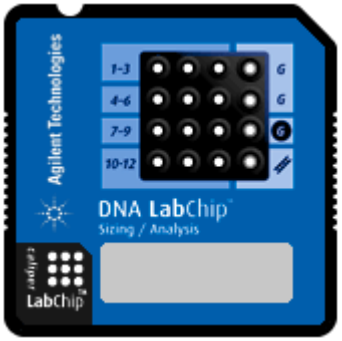
Do not force the lid closed. This may damage the cartridge.

d Carefully close the lid.

Electrophoretic assays: the electrodes in the cartridge fit into the wells of the chip.

Flow cytometric assays: the adapter with the gasket in the cartridge fits onto the priming well of the chip.

When the chip is detected, the image on the *Instrument* tab changes to a chip.



If the chip is not detected, open and close the lid again.

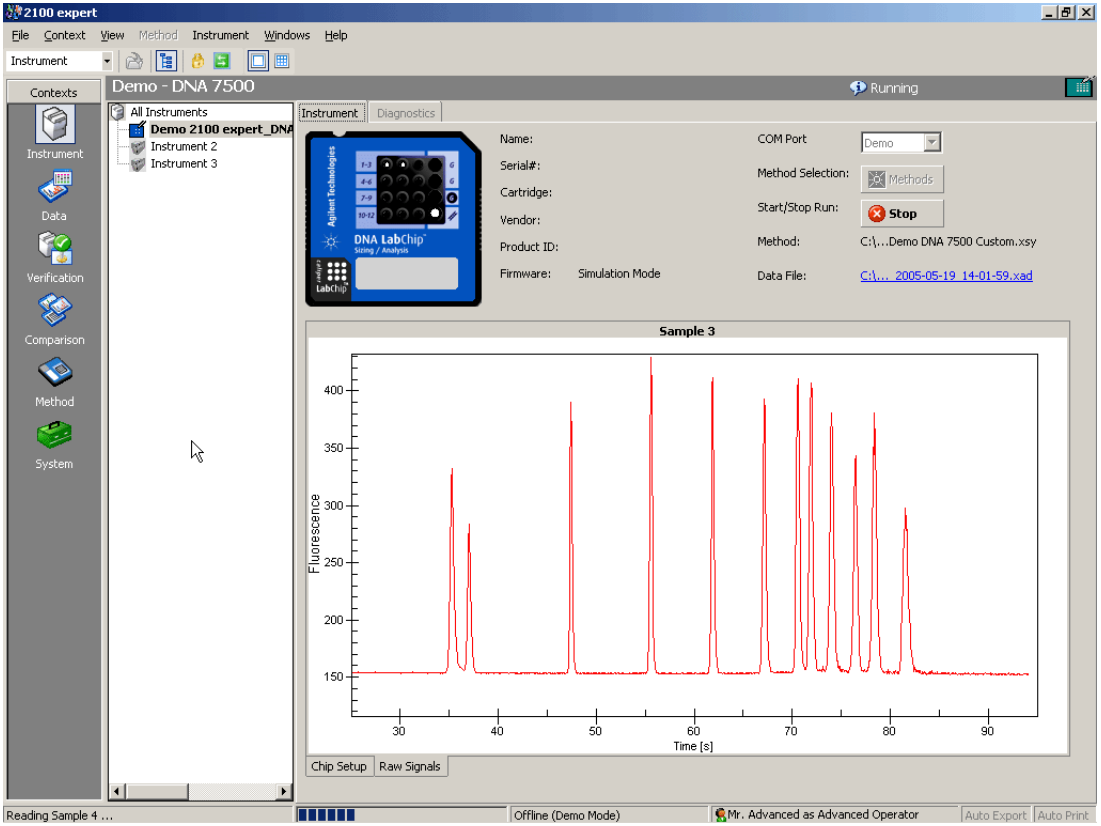
NOTE

If the *AutoRun* option is active, the chip run starts automatically once a chip has been inserted and the lid has been closed.

---

6 On the *Instrument* tab, click the *Start* button.

The chip run starts. The *Raw Signals* sub-tab shows an electropherogram of the currently measured sample. The name of the sample is displayed above the graph. The graph is a "live" plot of the migration time against fluorescence units (raw data, including background fluorescence, for example).

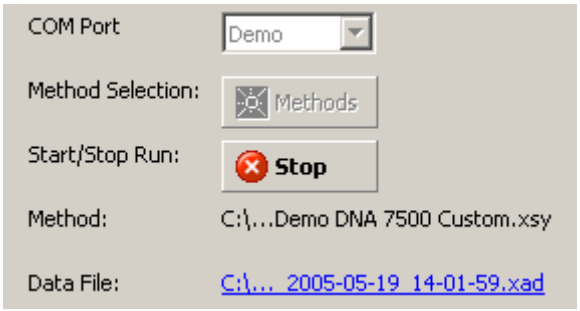


The number of the sample that is currently being measured is indicated on the information bar:

The status bar at the bottom of the window shows the measurement progress for the chip run and the COM port number used for data acquisition.

During the chip run, you can do the following:

- View the chip data file in the *Data* context by clicking on the name of the *Data File*:

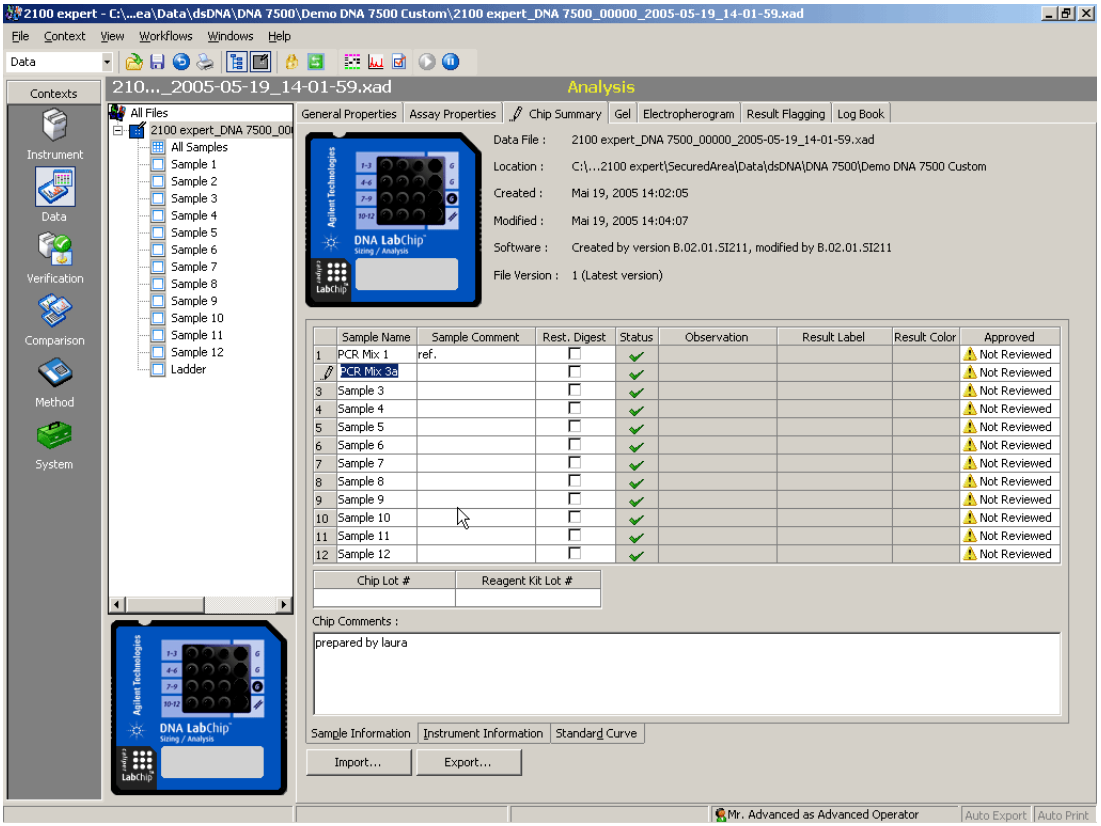


- Switch to any other context. For example, you can evaluate any chip data file in the *Data* context, or compare samples in the *Comparison* context.
- If necessary, abort the chip run by clicking on the *Stop* button.

  All data that was collected up to the stop point will be saved.

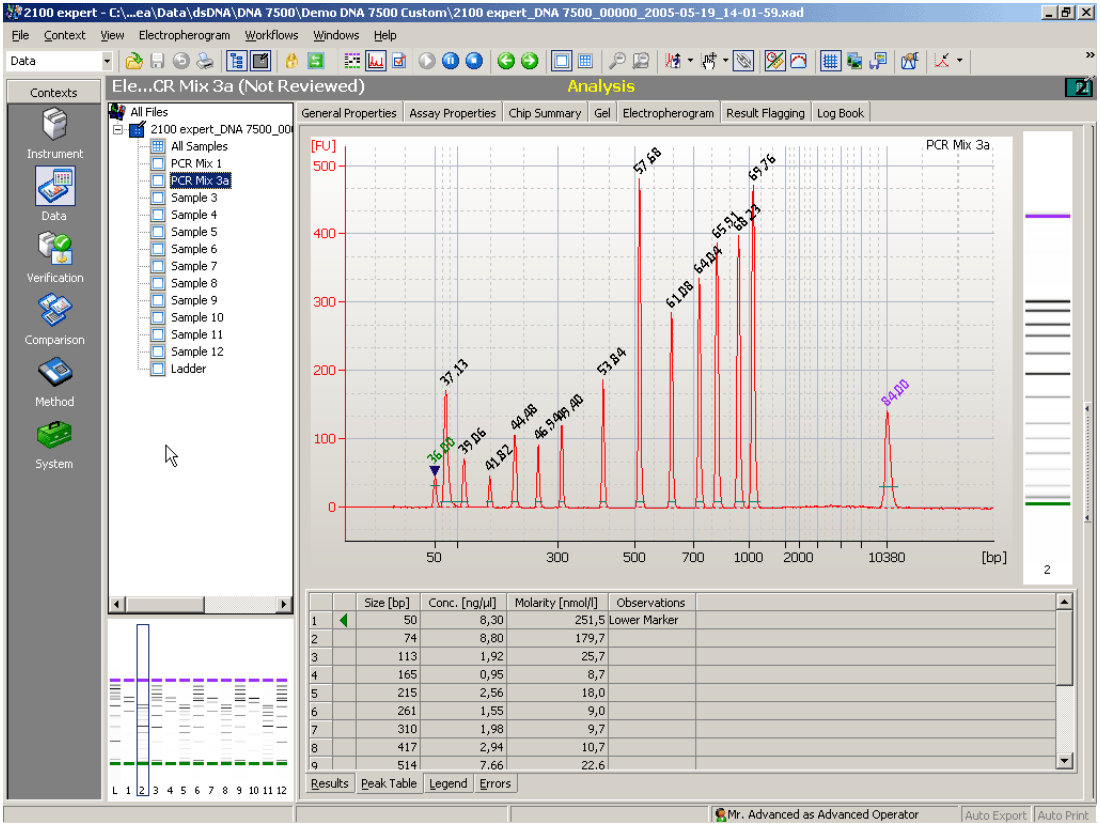
Viewing the Measurement Results

To view the results, switch to the *Data* context. The data file that has just been generated by your chip run is displayed. The *Chip Summary* tab shows information on your chip data file, and lets you enter comments regarding chip, samples, and study.



7 In the tree view panel, click any sample name or the ladder.

This selects the *Electropherogram* tab, which displays a data plot of size/migration time versus fluorescence intensity.



Peaks have automatically been detected, and their characteristics such as size, concentration, purity, or molarity have been calculated and are shown in the *Peak Table* at the bottom of the window.

What You Can do When the Measurement is Finished

When the measurement is finished, you can:

- Document your chip run by entering sample names, chip comments, and study information, for example.
- Evaluate the measurement results by analyzing gel-like images and electropherograms (electrophoretic assays), or histograms and dot plots (flow cytometric assays):
  - "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 81
  - "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 211
- Print the results to document them on paper or an electronic format, such as HTML or PDF.

  See "Printing Reports" on page 286.
- Export the results or parts of them for further evaluation in other applications.

  See "Exporting Data" on page 277.
- Compare the results with the results of other chip runs in the *Comparison* context. See "Comparing Samples from Different Electrophoretic Chip Runs" on page 143.
- Insert the next chip in the bioanalyzer and start a new chip run.

Looking at 2100 Expert

Before you start running assays on the Agilent 2100 bioanalyzer, you should familiarize yourself with the 2100 expert software:

- "Introduction to the Key Features of the 2100 expert" on page 29
- "Starting 2100 Expert" on page 31
- "2100 Expert Work Area" on page 32
- "Closing 2100 Expert" on page 43

Introduction to the Key Features of the 2100 expert

The Agilent 2100 expert is characterized by the following key features:

- 2100 expert provides a single software platform with a common user interface for running, analyzing, evaluating, presenting, and comparing DNA, RNA, protein *and* cell parameters.
- 2100 expert is installed in one go. After installation, the functionality for electrophoretic and flow cytometric assays can be activated separately with license keys.
- 2100 expert provides an optional security pack that needs to be ordered separately as G2949CA and is then activated with a license key. This security pack activates user management functions and electronic signature to meet the Food and Drug Administation (FDA) requirements (21 CFR Part 11).
- 2100 expert provides detailed installation verification and system verification tests on both the bioanalyzer hardware and software.
- 2100 expert allows having multiple chip data and/or assay files open at the same time.
- 2100 expert features a new integrated data evaluation tool (*Comparison* context) allowing comparison of measurement results (of same assay class) from different chips directly. A separate data evaluation tool is no longer necessary.
- 2100 expert features improved integration including manual integration (available for DNA and Protein assays only).
- 2100 expert allows color-coded result flagging with pre-defined or custom result flagging rules. Flagging rules can be applied to measurement results.
- 2100 expert now has customizable result tables and gel-like images.

- 2100 expert has improved instrument control. Two bioanalyzers can be controlled at one time. It is possible to run measurements as well as diagnostics tests on two bioanalyzers *at the same time*.
- 2100 expert has improved printing and reporting functions.
- 2100 expert has extended instrument diagnostics functionality.

Starting 2100 Expert

To start 2100 expert:

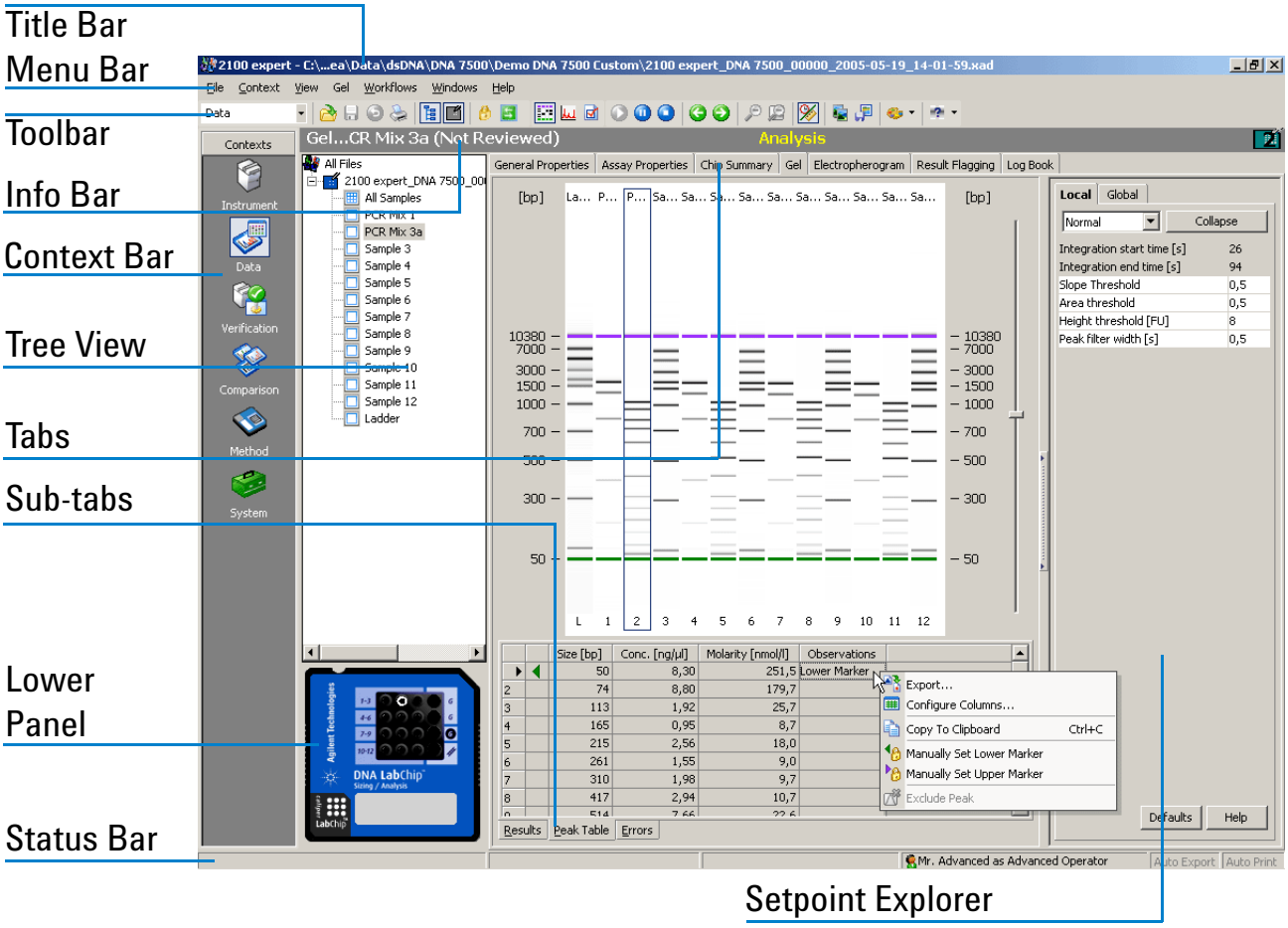
1 Go to your desktop and double-click the following icon:

– OR –

From the Windows *Start* menu, select *Programs* > *Agilent 2100 Bioanalyzer* > *2100 expert*.

2100 Expert Work Area

The 2100 expert software has standard elements such as pull-down menus and toolbars, and the main working area, which contains several tabs, some of which have sub-tabs. The 2100 expert work area has the following regions (demonstrated at the *Data* context):



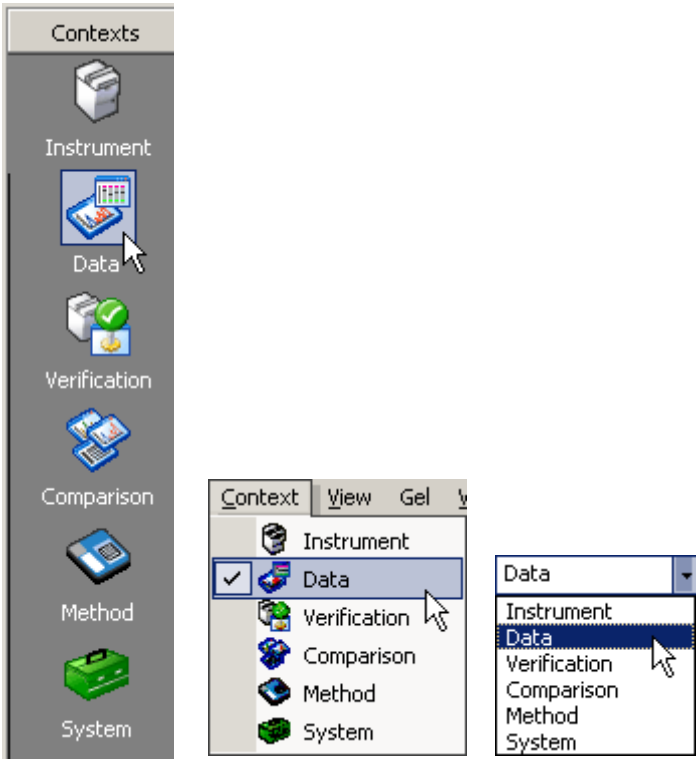
The 2100 expert software can be operated in six modes, called “contexts”:

- Instrument Context
- Data Context
- Verification Context
- Comparison Context
- Assay Context
- System Context

NOTE

The contexts work independent from each other regarding their data. This means, for example, that you can review data and run measurements at the same time.

Using the *Contexts* bar, the *Context* menu, or the selection list in the *toolbar*, you can switch between the contexts:



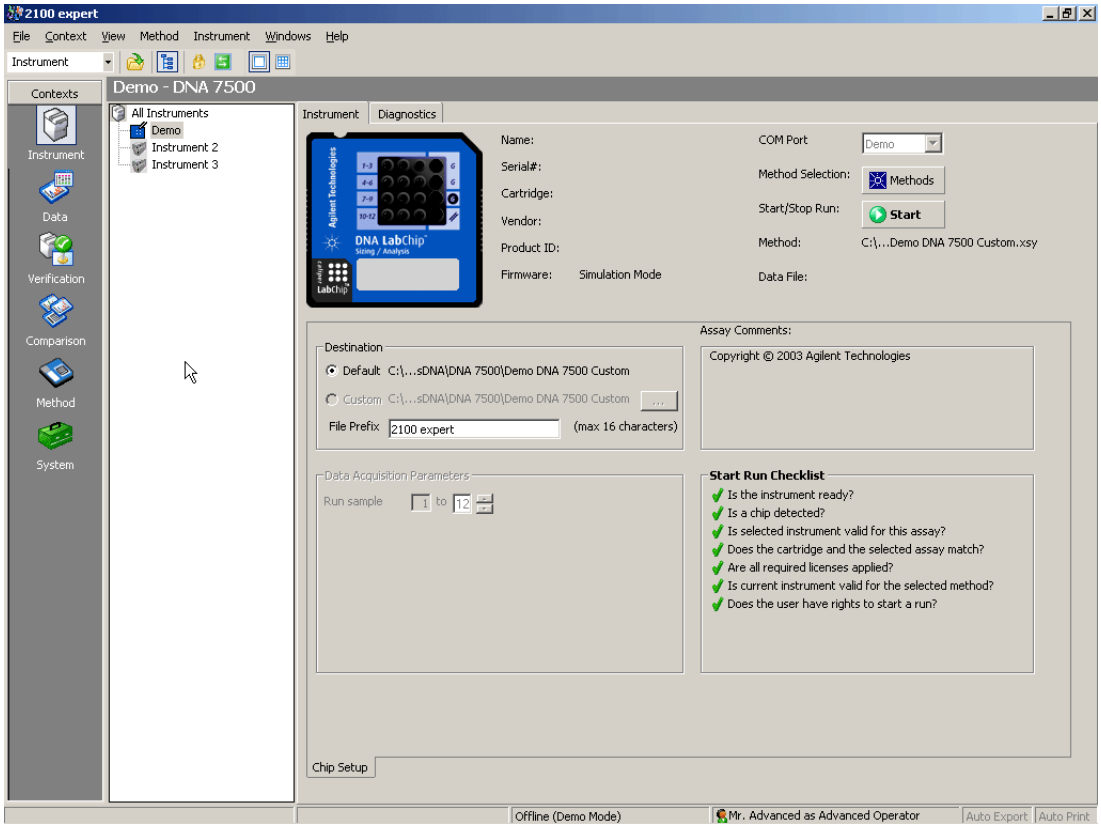
NOTE

Menus, toolbars, the tree view, and the main working area (tabs) significantly change when you switch between the contexts.

An introduction to the six contexts is given in the following.

Instrument Context

On startup, 2100 expert enters the *Instrument* context, where you can run DNA, RNA, protein or cell assays by selecting an assay file and starting the chip run—provided that the bioanalyzer is properly connected, a chip is inserted, and the bioanalyzer lid is closed.



NOTE

If two bioanalyzers are connected to your PC, you can run both in parallel.

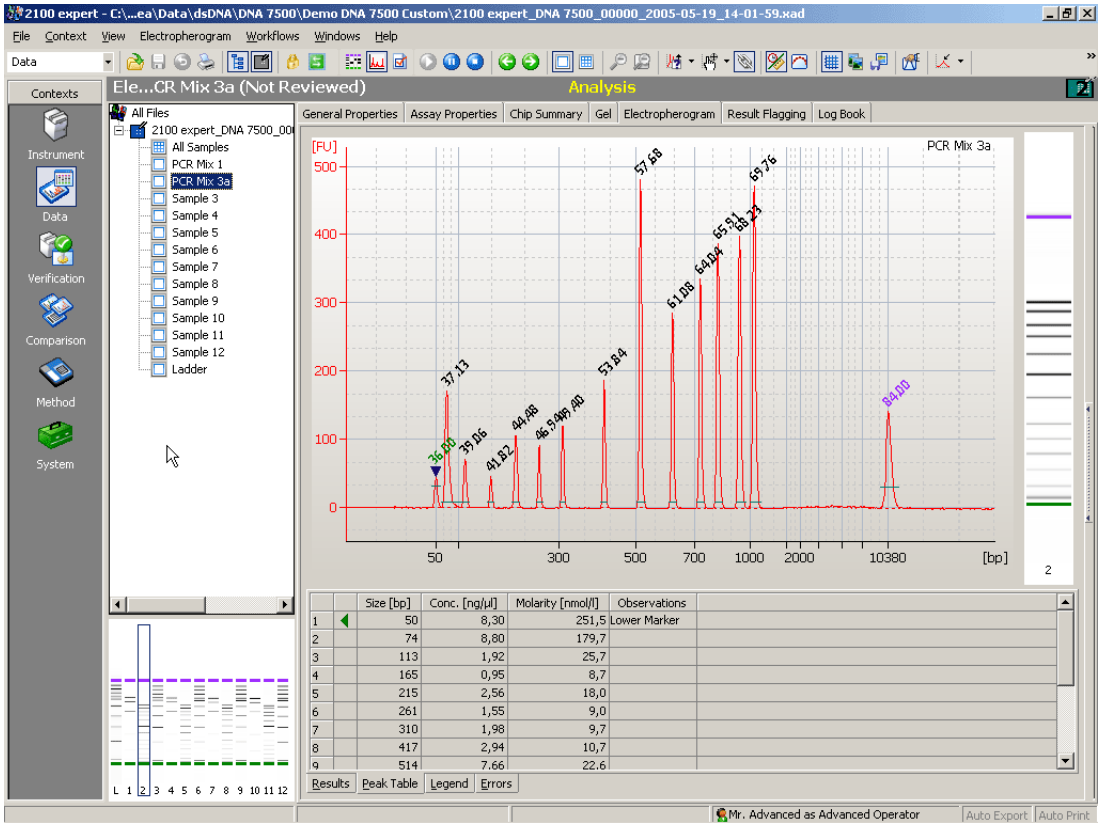
During the chip run(s), you can view the status of the bioanalyzer(s): instrument information and real time acquisition data.

In the *Instrument* context, it is also possible to run hardware diagnostic tests on all connected bioanalyzers. Refer to "Running Instrument Diagnostics" on page 316 for details.

Data Context

In the *Data* context, you can

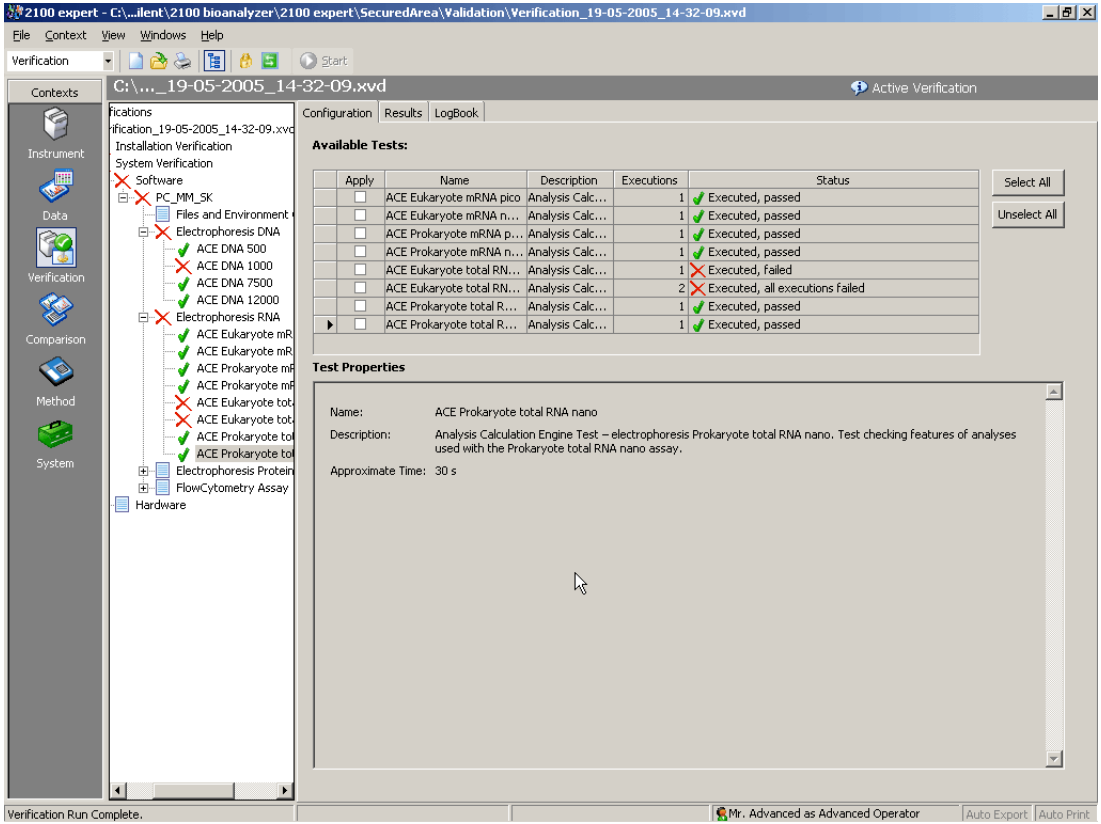
- view, analyze, and evaluate the results of your chip runs that are presented as electropherograms, gel-like images, histograms, dot plots, and result tables.
- export and print the results of your chip runs.



The measurement data is stored in .xad files.

Verification Context

The *Verification* context is used to run and document qualification tests.



For both the bioanalyzer hardware and software tests can be run for:

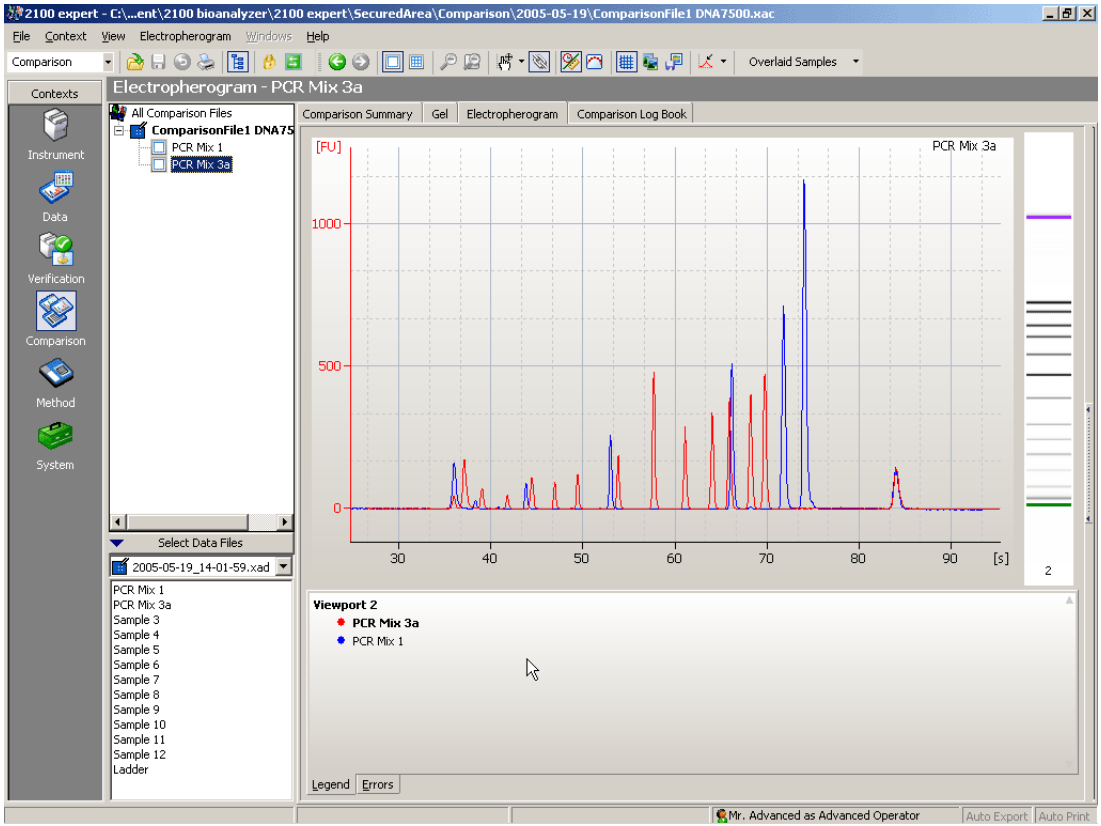
- Installation verification
- System verification

Verification results are automatically saved in .xvd files. You can re-open .xvd files to review verification results.

For details, refer to “Performing Verifications” on page 325.

Comparison Context

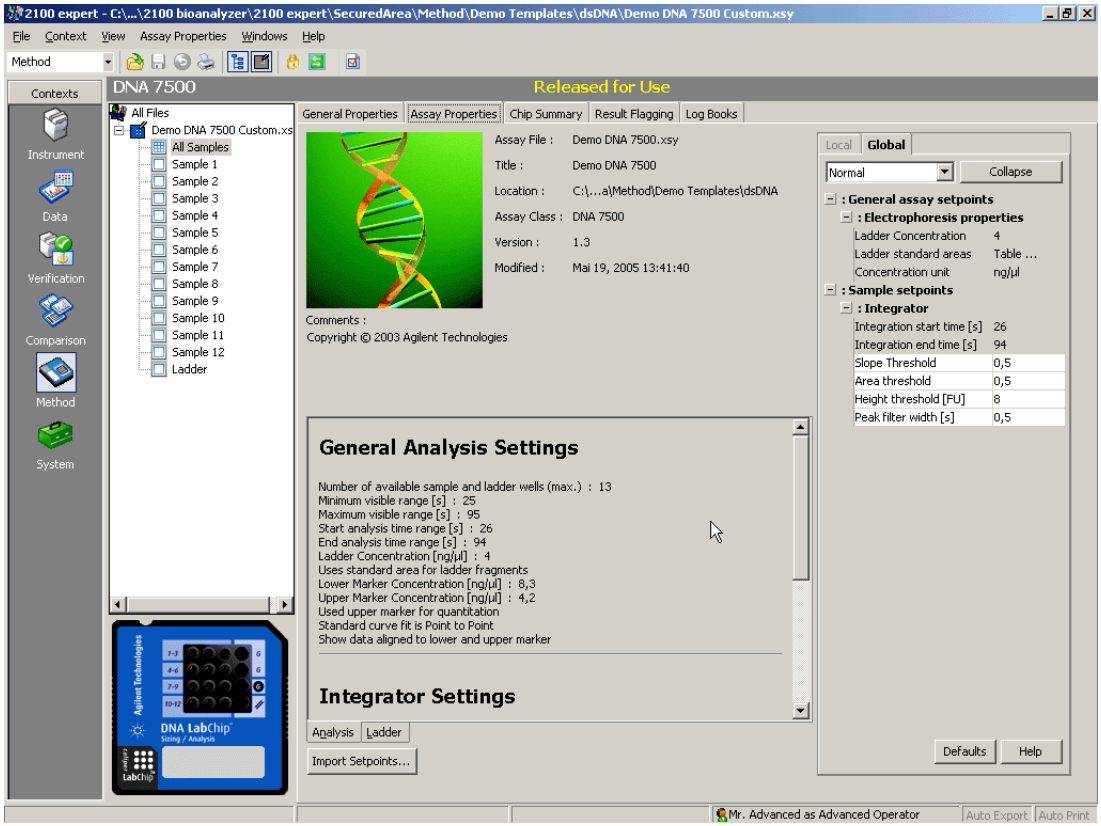
In the *Comparison* context, you can open multiple electrophoretic chip data files and compare samples of the same assay class (DNA 1000, for example). It is possible to overlay electropherograms recorded by the bioanalyzer and compare the results.



Comparison results can be saved in .xac files. You can re-open .xac files to review the comparison results and to add further samples for comparison.

Assay Context

In the *Assay* context, you can create your own assays based on Agilent templates by modifying certain data (for example, data analysis setpoints).

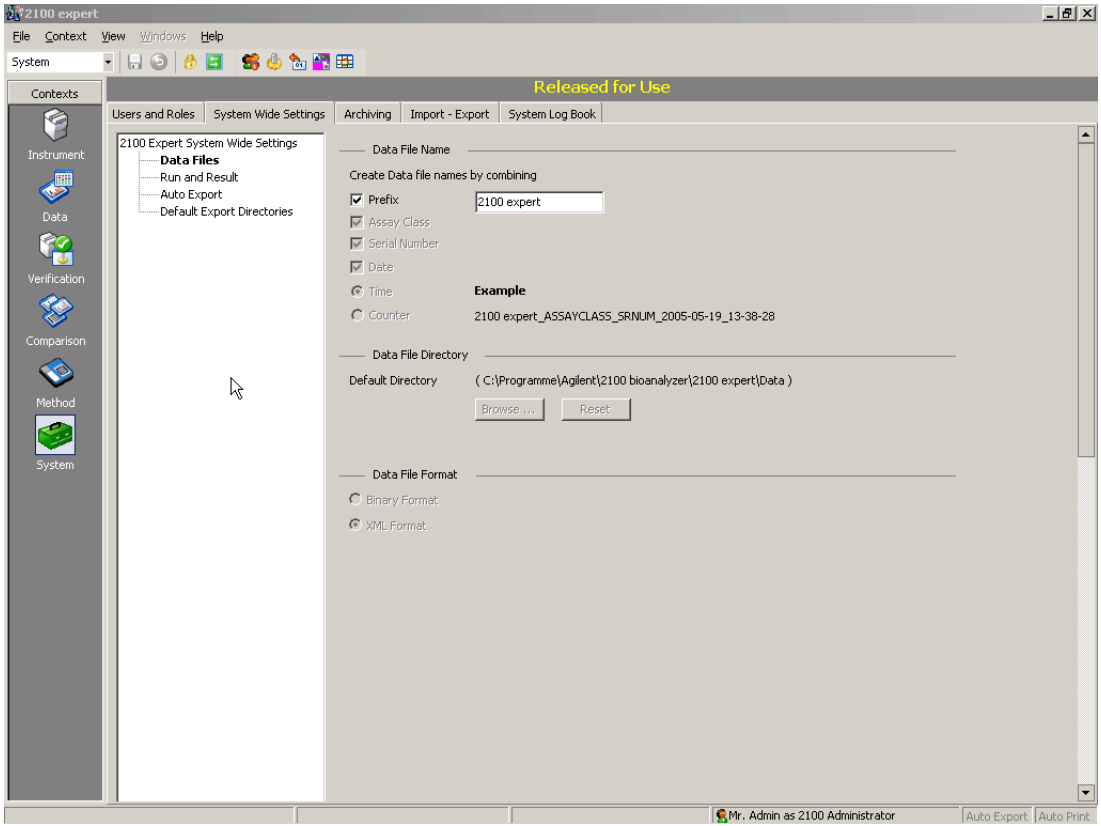


Assays are stored as .xsy files.

System Context

In the *System* context, you can

- define *System Wide Settings* for the 2100 expert software such as settings for default file names and directories, signal colors, or auto export functions
- view the contents of the *System Log Book*



Closing 2100 Expert

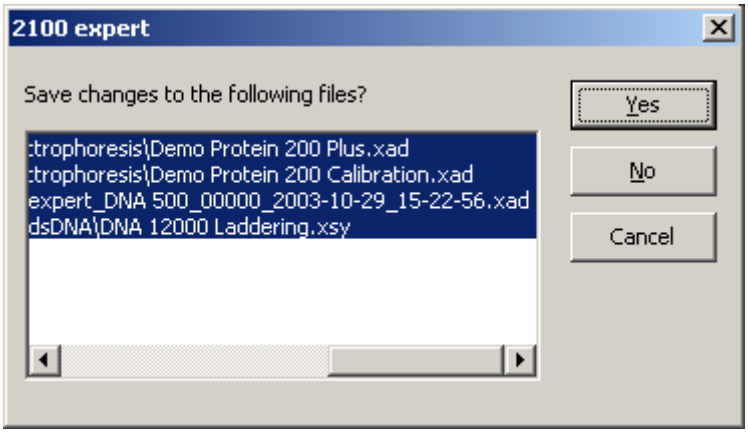
To close the 2100 expert software:

1 From the *File* menu, select *Exit*.

If a chip run is in progress, the following message appears:

Click *OK* and wait until the chip run is complete.

If there are unsaved files open, the following dialog box appears:



NOTE

This dialog box may also appear if you try to switch between contexts while there is unsaved data.

2 Click *Yes* to save the changes to the selected files and continue quitting 2100 expert.

If you want to save changes only to particular files, select these files in the list by single-clicking them. By default, all files with unsaved changes are selected.

If you click *No*, 2100 expert quits without saving any changes.

If you do not want to quit 2100 expert at this time, click *Cancel* to return to your 2100 expert session without saving anything.

After you have confirmed the messages, 2100 expert quits.

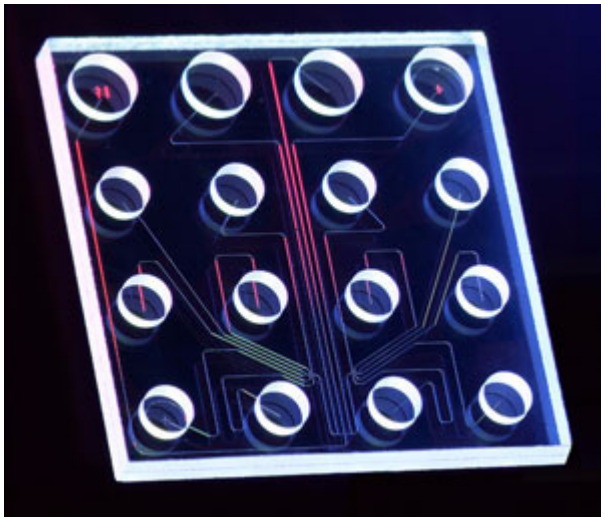
Running and Evaluating Electrophoretic Assays

For running and evaluating electrophoretic assays you need to know the following:

- "Principles of Nucleic Acid and Protein Analysis on a Chip" on page 46
- "Preparing and Running an Electrophoretic Assay" on page 50
- "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 81
- "Result Flagging" on page 152

Principles of Nucleic Acid and Protein Analysis on a Chip

The electrophoretic assays are based on traditional gel electrophoresis principles that have been transferred to a chip format. The chip format dramatically reduces separation time as well as sample and reagent consumption. The system provides automated sizing and quantitation information in a digital format. On-chip gel electrophoresis is performed for the analysis of DNA, RNA and proteins.



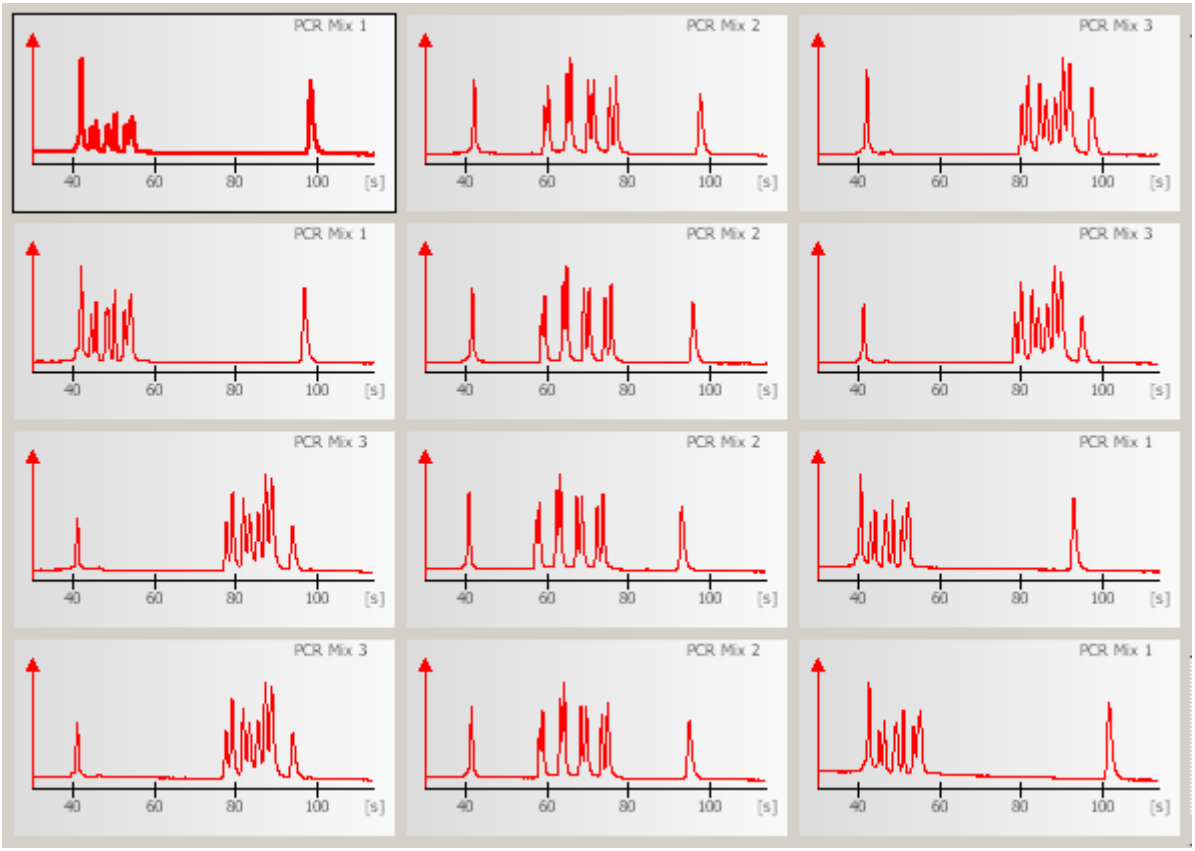
The chip accommodates sample wells, gel wells and a well for an external standard (ladder). Micro-channels are fabricated in glass to create interconnected networks among these wells. During chip preparation, the micro-channels are filled with a sieving polymer and fluorescence dye. Once the wells and channels are filled, the chip becomes an integrated electrical circuit. The 16-pin electrodes of the cartridge are arranged so that they fit into the wells of the chip. Each electrode is connected to an independent power supply that provides maximum control and flexibility. Charged biomolecules like DNA,

RNA, or protein/LDS micells are electrophoretically driven by a voltage gradient—similar to slab gel electrophoresis. Because of a constant mass-to-charge ratio and the presence of a sieving polymer matrix, the molecules are separated by size. Smaller fragments are migrating faster than larger ones. Dye molecules intercalate into DNA or RNA strands or protein/LDS micells. These complexes are detected by laser-induced fluorescence. Data is translated into gel-like images (bands) and electropherograms (peaks). With the help of a ladder that contains components of known sizes, a standard curve of migration time versus fragments size is plotted. From the migration times measured for each fragment in the sample, the size is calculated. Two markers (for RNA only one marker) are run with each of the samples bracketing the overall sizing range. The "lower" and "upper" markers are internal standards used to align the ladder data with data from the sample wells. This is necessary to compensate for drift effects that may occur during the course of a chip run.

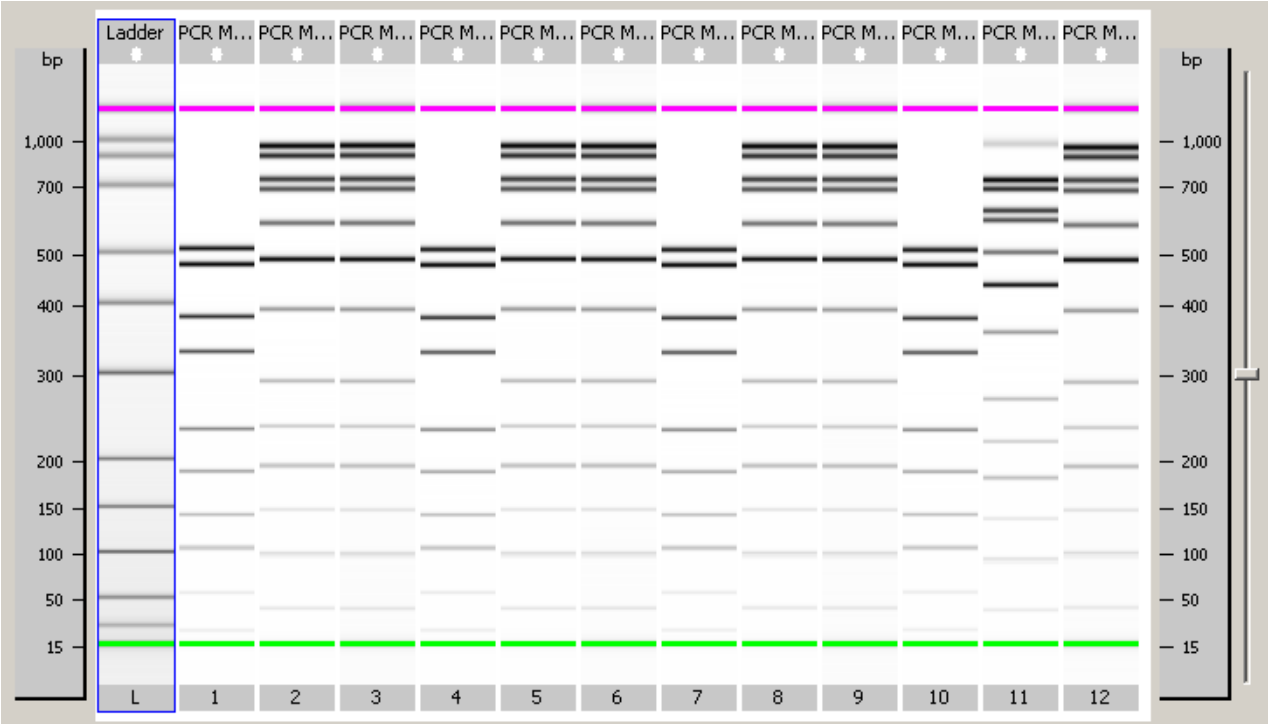
For DNA and protein assays, quantitation is done with the help of the upper marker. The area under the upper marker peak is compared with the sample peak areas. Because the concentration of the upper marker is known, the concentration for each sample can be calculated. Besides this relative quantitation, an absolute quantitation is available for protein assays, using external standard proteins.

For RNA assays, quantitation is done with the help of the ladder area. The area under the ladder is compared with the sum of the sample peak areas. The area under the "lower" marker is not taken into consideration. For total RNA assays, the ribosomal ratio is determined, giving an indication on the integrity of the RNA sample. Additionally, the RNA integrity number (RIN) can be utilized to estimate the integrity of total RNA samples based on the entire electrophoretic trace of the RNA sample, including the presence or absence of degradation products.

The 2100 expert software plots fluorescence intensity versus size/migration time and produces an electropherogram for each sample:



The data can also be displayed as a densitometry plot, creating a gel-like image:



Preparing and Running an Electrophoretic Assay

An electrophoretic chip run requires the following steps:

1. Switch on the Agilent 2100 bioanalyzer and start the 2100 expert software.

   See "Starting 2100 Expert" on page 31.

2. Select an electrophoretic assay.

   See "Selecting an Electrophoretic Assay for a Chip Run" on page 51.

3. Prepare reagents, chip, and samples.

   See "Preparing Samples, Reagents, and Chips for Electrophoretic Assays" on page 54 and the appropriate *Application Note* or *Reagent Kit Guide*.

4. Load the chip into the bioanalyzer.

   See "Loading the Electrophoresis Chip into the Bioanalyzer" on page 58.

5. Start the chip run.

   See "Running an Electrophoretic Assay" on page 64.

When the chip run has finished, you can:

- Have a first look at the results (see "Displaying the Measurement Results (Electrophoresis)" on page 72).
- Document the chip run (see "Entering Chip, Sample, and Study Information" on page 69).
- Analyze and evaluate the results:
  - See "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 81.
  - See "Result Flagging" on page 152.

Selecting an Electrophoretic Assay for a Chip Run

To select an assay:

1 Switch to the *Instrument* context.

2 In the *Tree View Panel*, select the bioanalyzer you want to use.

In the upper left of the *Instrument* tab, an icon shows the status of the bioanalyzer. You should see one of the following icons (lid open/closed), indicating that the bioanalyzer is detected by the system:

3 If you do not see one of these icons, check that the bioanalyzer is switched on and properly connected:

- Check the *COM port* setting.
- Make sure the bioanalyzer is physically connected to the PC (over the serial interface).
- Check the power connection.
- Check the power switch.

If you need additional help, please refer to the *Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide*.

4 Select an assay for the chip run.

On the *Instrument* tab, click the *Assay* button.

– OR –

Click the *Assays* menu.

Both will open the *Assays* menu, allowing you to select an assay from the submenus.

– OR –

Select *File* > *Open File to Run*. This opens a dialog box, allowing you to load either an assay (.xsy) or a chip data file (.xad).

The type of assay you have to select depends on the required measurement and the Reagent Kit you use to prepare your samples. Details on these assays are described in the *Application Notes* available for each assay and in the *Reagent Kit Guide*.

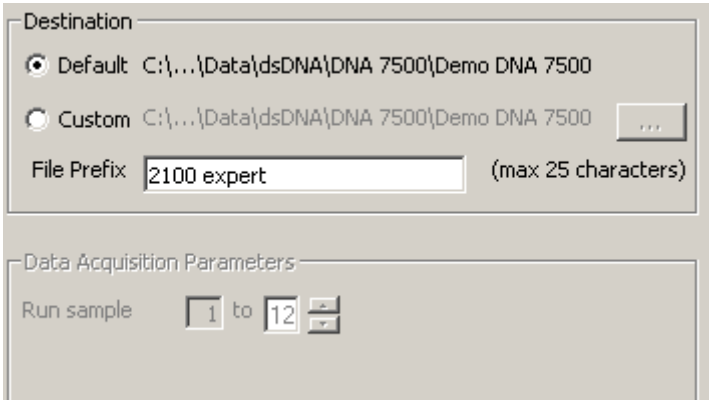
5 Select the desired assay, *DNA 1000*, for example.

The assay is loaded and its name appears on the *Information Bar*:

NOTE

After a chip run, the results can be evaluated using parameters from a different electrophoretic chip data file (.xad) of the same assay type (DNA 1000 in this example). Refer to "Importing Data Analysis Setpoints" on page 273.

6 Select a *Destination* for the chip data file (.xad) that will be generated as the result of the chip run. You can also specify a custom *File Prefix* for this file.



7 Under *Data Acquisition Parameters*, enter the number of samples you want to have measured.

The total number of samples that can be measured varies with the type of assay selected. With DNA and RNA Nano assays, 12 samples may be run; with RNA Pico assays, 11 samples may be run; and with Protein assays, the maximum number of samples is 10. When preparing the chip (see "Preparing Samples, Reagents, and Chips for Electrophoretic Assays" on page 54), keep in mind that you have to follow the sequence of the sample wells. For example, if you want to measure only 3 samples, you have to fill the wells 1, 2, and 3 of your chip.

Preparing Samples, Reagents, and Chips for Electrophoretic Assays

Before you can load a chip, you have to prepare the samples and reagents. To find out how to prepare the samples and reagents, refer to the various *Reagent Kit Guides* available for each LabChip kit. Please refer to these documents for further information and analytical specifications.

In general, preparing an electrophoretic assay involves the following steps:

- Check that you have everything that is listed in the appropriate Reagent Kit Guide.

  Be aware that there can be small but important differences between the different assays even for the same type of molecules (for example, between DNA 1000 and DNA 7500 assays).
- Make sure you are familiar with the essential measurement practices (see next page).
- Before running the first RNA assay: decontaminate the electrodes.
- Prepare all the reagent mixtures (for example, the gel-dye mix).
- Load the gel-dye mix using the priming station.
- Load the DNA/RNA marker solution and buffer.
- Load the destaining solution for protein assays.
- Load the chip with ladder and samples.

Essential Measurement Practices (Electrophoretic Assays)

General:

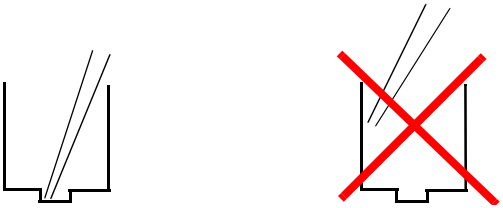
WARNING

Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.

WARNING

No data is available addressing the mutagenicity or toxicity of the dye/DMSO reagent. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO mixtures should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling DMSO mixtures.

- Handle and store all reagents according to the instructions given in the *Reagent Kit Guides*.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results due to the formation of a bubble on the bottom of the well.



- Protect dye and gel-dye mix from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Use a new syringe and electrode cleaner with each new LabChip kit.
- Do not touch the Agilent 2100 bioanalyzer during a chip run and never place it on a vibrating ground.
- Keep all reagents and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.
- Use loaded chips within 5 minutes. Reagents might evaporate, leading to poor results.

RNA Assays:

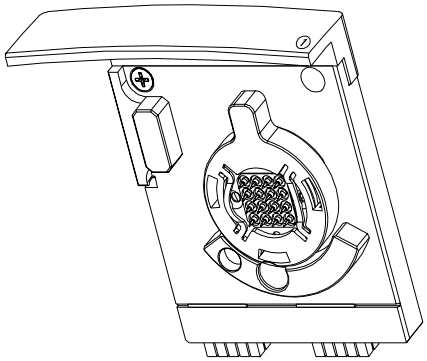
- Always wear gloves when handling RNA, and use RNase-free tips, microfuge tubes and water.
- It is recommended to denature all RNA samples and RNA ladder by heat before use (70 °C, 2 minutes).
- Always vortex the dye concentrate for 10 seconds before preparing the gel-dye mix.

Protein Assays:

- Store Protein sample buffer at -20 °C upon arrival. Keep the vial in use at 4 °C to avoid freeze-thaw cycles.
- Allow the dye concentrate to equilibrate to room temperature for 20 minutes before use, to make sure the DMSO is completely thawed. Protect the dye from light during that time. Vortex before use.
- Allow all other reagents to equilibrate to room temperature for 10 minutes before use.
- Use 0.5 ml tubes to denature samples. Using larger tubes may lead to poor results, caused by evaporation.

Loading the Electrophoresis Chip into the Bioanalyzer

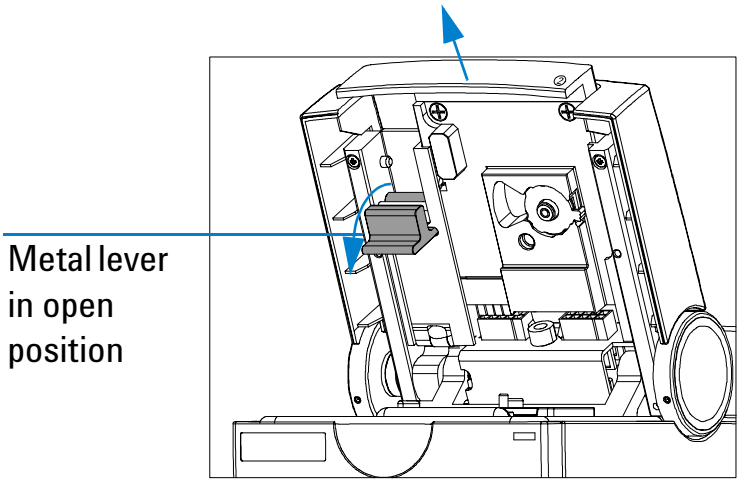
The Agilent 2100 bioanalyzer uses different cartridges for electrophoretic and flow cytometric assays. For electrophoretic measurements, the *electrode cartridge* is required.



The electrode cartridge contains 16 electrodes that fit into the wells of DNA, RNA, and Protein chips. Each electrode in the cartridge has an individual power supply. All electrophoretic assays (DNA, RNA, and Protein) require an electrode cartridge. The electrode cartridges will either have an engraved “1” at the front, or will have no engraving at all. Cartridges with a different number are not electrode cartridges.

If the bioanalyzer is set up for flow cytometric assays, but you want to run electrophoretic assays, proceed as follows:

1 Open the lid and pull down the metal locking lever into the open position as shown in the figure below.



The cartridge is pushed out.

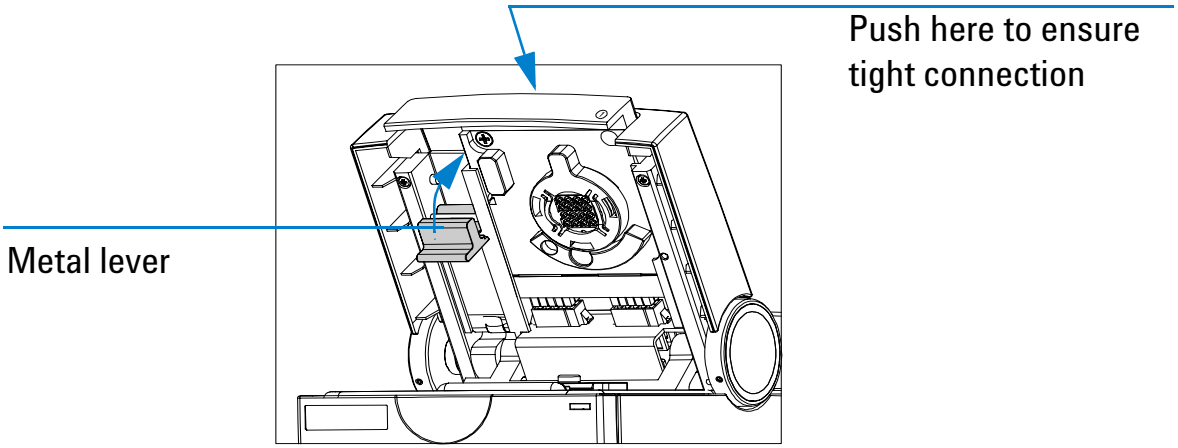
2 Gently pull the cartridge out of the lid.

NOTE

Store the pressure cartridge in the provided box.

---

3 Slide the electrode cartridge into the lid as shown below.



CAUTION

Do not touch the electrodes while the cartridge is in the Agilent 2100 bioanalyzer. The electrodes and the high voltage power supplies can be damaged.

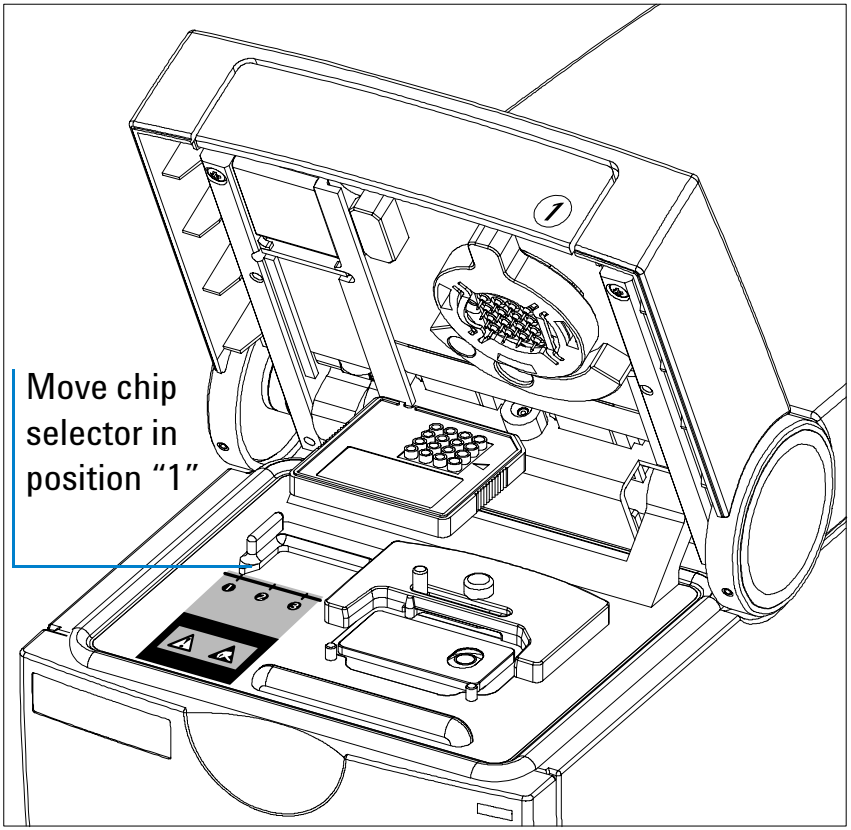
4 Push the metal front of the cartridge to ensure a tight connection.

5 Push the metal locking lever into the flat (closed) position.

To load the prepared chip into the Agilent 2100 bioanalyzer:

1 Open the lid and remove any chip.

2 Adjust the chip selector to position “1” as shown in the following figure.



To avoid using incompatible chips and cartridges, a chip selector is installed in the bioanalyzer. This ensures that the chip matches to the installed cartridge.

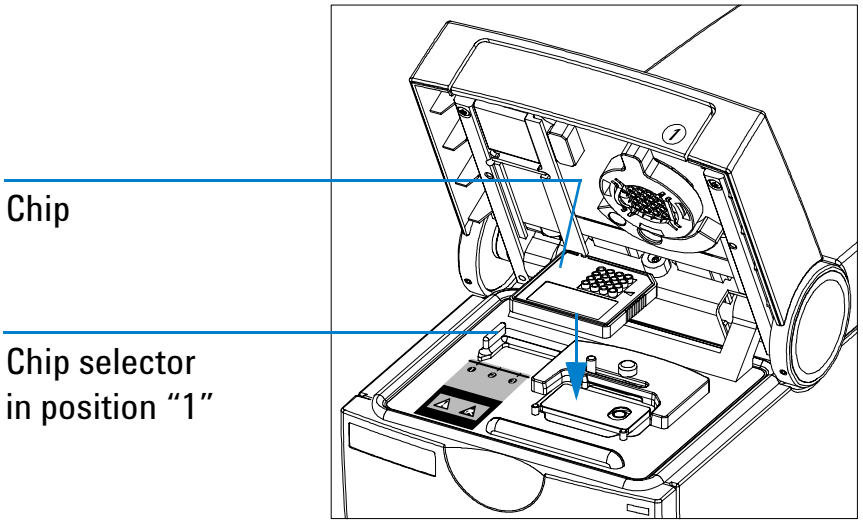
These steps are required for inserting DNA, RNA, and Protein chips in the bioanalyzer.

CAUTION

Do not force the chip selector handle when a chip is inserted in the bioanalyzer.

3 Place the prepared chip into the receptacle.

The chip fits only one way. Do not force it into place.

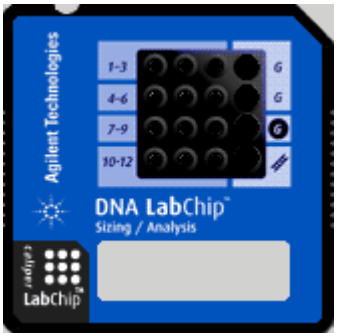


4 Carefully close the lid.

CAUTION

Do not force the lid closed. This may damage the cartridge. If the lid does not close completely, check that the cartridge and chip are inserted properly, and the chip selector is in the correct position.

When the chip is detected, the image on the *Instrument* tab changes to a chip.



If the chip is not detected, open and close the lid again.

NOTE

The displayed image depends on the assay selcted in the software, not the type of chip inserted. If you would like to run a DNA chip but a protein chip appears, you have selected the wrong assay.

NOTE

If the *AutoRun* option is active, the chip run starts automatically once a chip has been detected and the lid has been closed.

NOTE

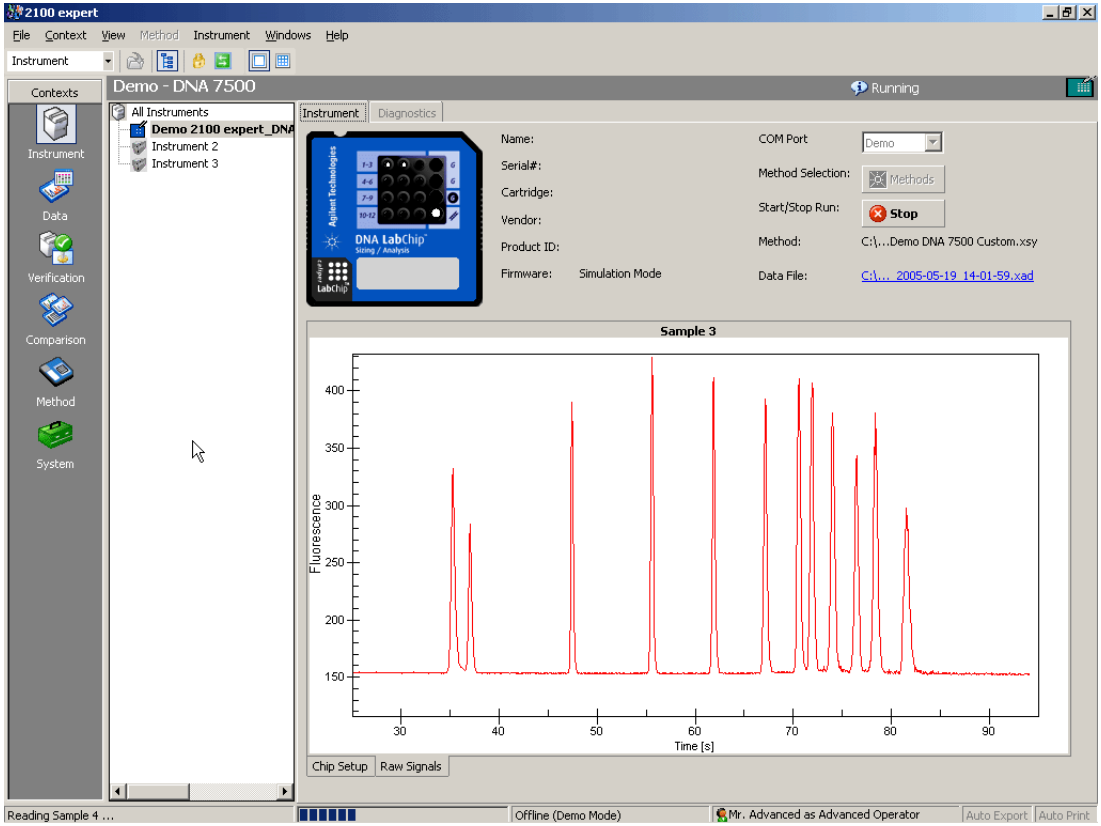
You can stop a chip run at any time, for example, if errors occurred or if you are not satisfied with the quality of the measurement results that you can observe during the chip run. See "Stopping a Chip Run" on page 67.

Starting the Chip Run

When you have loaded the chip, you can start the chip run:

1 On the *Instrument* tab, click the *Start* button.

The chip run starts. The *Raw Signals* sub-tab shows an electropherogram of the currently measured sample. The name of the sample is displayed above the graph. The graph is a "live" plot of the migration time against fluorescence units (raw data, including background fluorescence, for example).

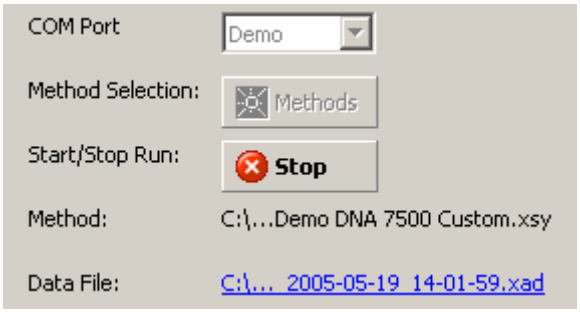


The number of the sample that is currently being measured is indicated on the information bar:

The status bar at the bottom of the screen shows the measurement progress for the chip run and the COM port number used for data acquisition.

During the chip run, you can do the following:

- View the chip data file in the *Data* context by clicking on the name of the *Data File*:



- Switch to any other context. For example, you can evaluate any chip data file in the *Data* context, or compare samples in the *Comparison* context.
- If necessary, abort the chip run by clicking on the *Stop* button.

  All data that was collected up to the stop point will be saved.

After the chip run is completed, you can:

- Switch to the *Data* context, where you can view, analyze, and evaluate the results of your chip run (see "Displaying the Measurement Results (Electrophoresis)" on page 72 and "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 81).
- Stay in the *Instrument* context and start a new assay, for example.

Stopping a Chip Run

You can stop a chip run at any time, for example,

- if the quality of the measurement results does not meet your expectations,
- if, for example, after three samples you already have the information you desired and you want to start another chip run.

NOTE

You *cannot* resume a stopped chip run.

NOTE

If you stop a chip run, automatic export (see "Exporting Chip Run Data Automatically" on page 280) and automatic print (see "How to Turn on and Configure Automatic Printing of Chip Run Reports" on page 291) does *not* take place.

To stop the assay:

1 Click the *Stop* button

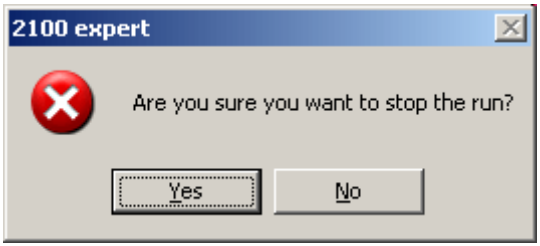
– OR –

Select *Stop* from the *Instrument* menu.

NOTE

Data acquisition of the current sample will be aborted.

The following message appears:



2 Click *Yes* to stop the chip run.

When the chip run is aborted, you can:

- Switch to the *Data* context, where you can view, analyze, and evaluate the results (if any) of your chip run (see "Displaying the Measurement Results (Electrophoresis)" on page 72 and "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 81).
- Stay in the *Instrument* context, where you can start the next chip run, for example.

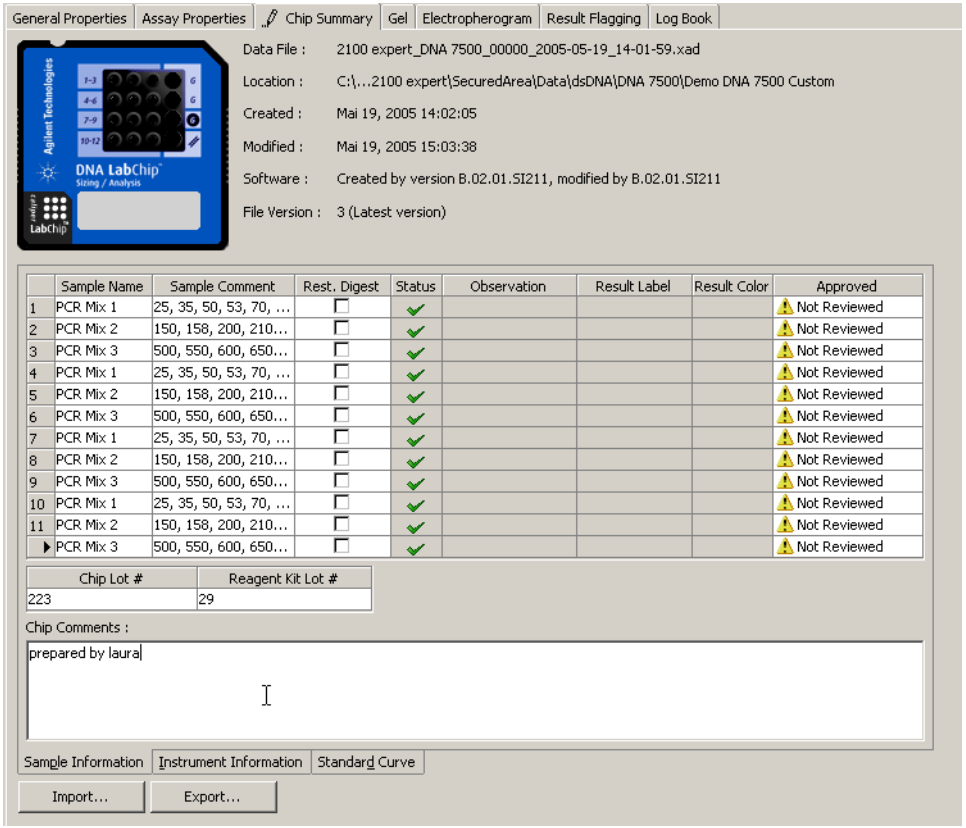
Entering Chip, Sample, and Study Information

During or after a chip run, you can document the run by entering information on chip, samples, and study.

1 In the *Data* context, select the *Chip Summary* tab.

2 On the *Sample Information* sub-tab, you can enter additional information such as sample names and comments.

On the *Study Information* sub-tab, you can enter information such as the name of the current study, the laboratory location, and the experimenter, for example.


General Properties
Assay Properties
Chip Summary
Gel
Electropherogram
Result Flagging
Log Book
Agilent Technologies
DNA LabChip
Sizing / Analysis
Data File : 2100 expert_DNA 7500_00000_2005-05-19_14-01-59.xad
Location : C:\...2100 expert\SecuredArea\Data\dsDNA\DNA 7500\Demo DNA 7500 Custom
Created : Mai 19, 2005 14:02:05
Modified : Mai 19, 2005 15:03:38
Software : Created by version B.02.01.SI211, modified by B.02.01.SI211
File Version : 3 (Latest version)
Sample Name | Sample Comment | Rest. Digest | Status | Observation | Result Label | Result Color | Approved
1 PCR Mix 1 25, 35, 50, 53, 70, ... Not Reviewed
2 PCR Mix 2 150, 158, 200, 210... Not Reviewed
3 PCR Mix 3 500, 550, 600, 650... Not Reviewed
4 PCR Mix 1 25, 35, 50, 53, 70, ... Not Reviewed
5 PCR Mix 2 150, 158, 200, 210... Not Reviewed
6 PCR Mix 3 500, 550, 600, 650... Not Reviewed
7 PCR Mix 1 25, 35, 50, 53, 70, ... Not Reviewed
8 PCR Mix 2 150, 158, 200, 210... Not Reviewed
9 PCR Mix 3 500, 550, 600, 650... Not Reviewed
10 PCR Mix 1 25, 35, 50, 53, 70, ... Not Reviewed
11 PCR Mix 2 150, 158, 200, 210... Not Reviewed
PCR Mix 3 500, 550, 600, 650... Not Reviewed
Chip Lot #
Reagent Kit Lot #
223
29
Chip Comments :
prepared by laura
Sample Information
Instrument Information
Standard Curve
Import...
Export...

NOTE

You may find some input fields already filled in, because chip, sample, and study information are taken over from the base assay or chip data file.

3 From the *File* menu, select *Save*.

TIP

You can import chip, sample, and study information from .txt or .csv files. This is especially helpful and time-saving, if you already have documented a similar chip run in another chip data file. Refer to "Importing Chip, Sample, and Study Information" on page 275 for details.

Displaying the Measurement Results (Electrophoresis)

You can view the measurement results of an electrophoretic chip run as electropherograms or gel-like images.

- You can display the electropherograms either one sample at a time, or all samples at the same time to get an overview of the chip run, for example, to see the progress of a reaction. See "How to Switch Between Single View and Grid View (Electropherograms)" on page 73.
- You can navigate through the samples. See "How to Navigate Through the Samples" on page 74.
- You can change the display of electropherograms and gel-like images to make details better visible. See "How to Change the Display of Electropherograms and Gel-like Images" on page 75.

How to Switch Between Single View and Grid View (Electropherograms)

To switch between single view and grid view:

1 From the *Electropherogram* menu, select *View Single Sample* or *View All Samples*.

– OR –

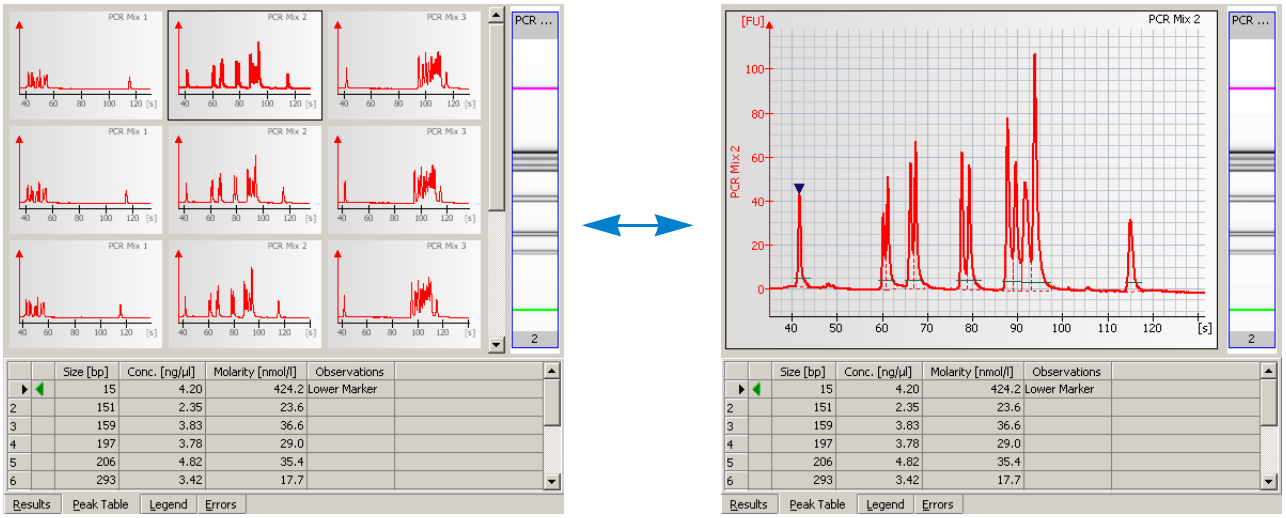
Click the *View Single Sample* or *View All Samples* button on the *Electropherogram* toolbar.

– OR –

Click the *All Samples* entry in the *Tree View Panel* to switch to the grid view, or any sample name to switch to the single view.

– OR –

Double-click any electropherogram the grid view to switch to single view:



How to Navigate Through the Samples

At any time—even during a chip run—you can scroll through all samples—either in electropherogram or gel view.

To navigate through samples using the *Tree View Panel*:

1. If the tree view is not visible, select *View* > *Tree View*.

   The tree view panel appears to the left of the tabs, and shows all chip data and assay files as nodes.

2. Click any sample name.

   Electropherogram view: the electropherogram of the selected sample is shown in single view

   Gel view: the lane of the gel-like image corresponding to the selected sample is highlighted.

To navigate through samples using the *Lower Panel*:

1. If the lower panel is not visible, select *View* > *Lower panel*.

   The lower panel appears in the lower left corner.

2. Electropherogram view: Click any lane of the small gel image.
   Gel view: Click any well on the chip icon.

To browse through samples:

1 From the *Electropherogram* or *Gel* menu, select *Next Sample* or *Previous Sample*.

– OR –

Click the *Next Sample* or *Previous Sample* button in the toolbar.

To switch between electropherogram and gel view:

1 Click the *Electropherogram* or *Gel* tab to display the results of the selected sample as an electropherogram or as a gel-like image.

How to Change the Display of Electropherograms and Gel-like Images

It is possible to change the display of electropherograms and gel-like images.

In electropherograms and gel-like images you can:

- zoom (enlarge or reduce using the mouse) the graphs to display details, for example.

In electropherograms, you can additionally:

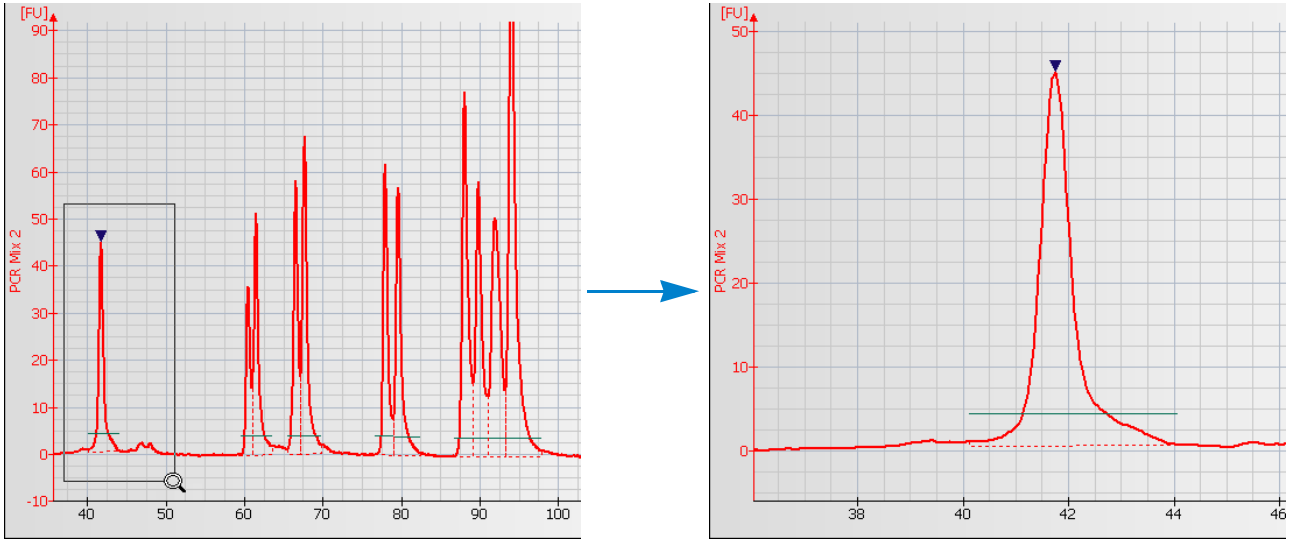
- show data points.
- pan and scale the graph using the mouse.
- change the background from a gray-to-white gradient to white.
- add a grid to the electropherograms.

In gel-like images, you can additionally:

- change the gel color.
- change order of gel lanes in gel like images.

To zoom into an electropherogram:

1. From the *Electropherogram* menu, select *Graph Mode* > *Zoom* (default setting).
2. Position the mouse pointer in the electropherogram.
3. Click and hold down the left mouse button.
   The mouse pointer changes its shape to a magnifying glass .
4. Drag the mouse.
   A rectangle shows the part of the an electropherogram to be enlarged.
5. Release the mouse button.



To pan and scale an electropherogram:

1 From the *Electropherogram* menu, select *Graph Mode* > *Pan* or *Scale*.

2 Position the mouse pointer in the electropherogram.

3 Click and hold down the left mouse button.

The mouse pointer changes its shape to a double-arrow or to a double crosshair.

4 Drag the mouse.

As you drag the mouse, the electropherogram curve moves in the drag direction (*Pan* mode), or the scales of the X and/or Y axes change (*Scale* mode).

5 Release the mouse button.

You can perform several zoom, pan and scale steps in a row.

To undo the last zoom, pan, or scale step:

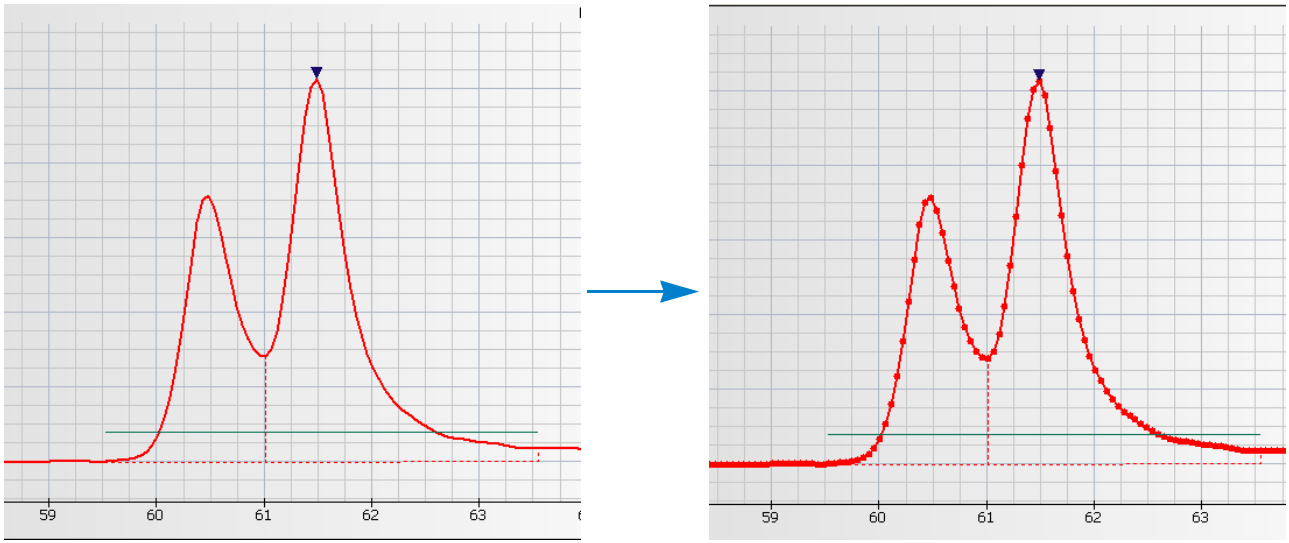
1 Click the *Undo Zoom* button or double-click in the electropherogram.

To undo all zoom, pan, and scale steps:

1 Click the *Undo All* button.

To display data points in an electropherogram:

1 From the *Electropherogram* menu, select *Show Data Points* or click the button in the toolbar.



Data points used to generate the graph are now shown as bullets. Data points are 0.05 seconds apart.

To remove the gray-to-white gradient from the background of an electropherogram:

1 From the *Electropherogram* menu, select *Show Gradient*.

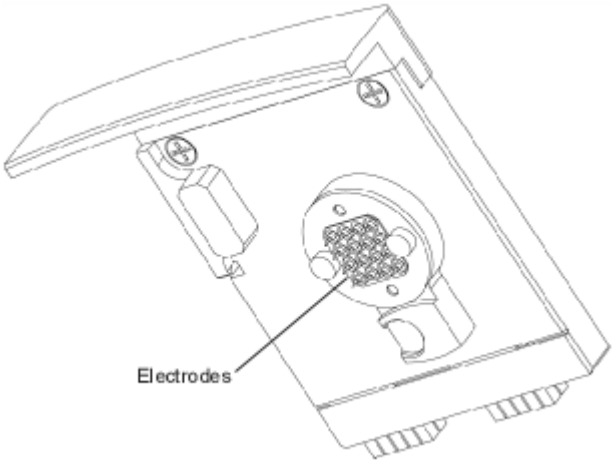
The color gradient disappears and a white background is displayed.

To show/hide the grid lines on an electropherogram:

1 From the *Electropherogram* menu, select *Show Grid*.

Cleaning the Electrodes after an Electrophoretic Assay

When the assay is complete, remove the used chip from the bioanalyzer and dispose of it according to the guidelines established by your laboratory safety officer. Remove the chip quickly to prevent a buildup of residues from the solutions on the electrodes.



Then perform the cleaning procedure to ensure that the electrodes are clean (i.e., no residues left from the previous assay). The cleaning procedures are described in detail in the appropriate *Reagent Kit Guide* and in the *Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide*.

Good Practices

- Empty and refill the electrode cleaner at regular intervals (e.g., every five assays).
- The electrode cleaner can be used for 25 assays.

CAUTION

Never use a cloth to clean the electrodes. Electrostatic discharge could damage the high-voltage power supplies.

CAUTION

Wet electrodes can cause severe damage to the on-board high voltage power supplies. Always make sure the electrodes are dry before inserting them into the bioanalyzer again.

Analyzing and Evaluating the Results of an Electrophoretic Assay

The purpose of electrophoretic assays is to separate sample components and determine their size, concentration, purity, or molarity. Results for a particular sample are calculated after all data for that sample has been read.

The steps in data analysis differ depending on the type of assay in use:

- "Data Analysis: DNA" on page 82
- "Data Analysis: RNA and Cy5-Labeled Nucleic Acids" on page 87
- "The RNA Integrity Number (RIN)" on page 90
- "Data Analysis: Protein" on page 104
- "Smear Analysis" on page 112

Further steps in analysis are:

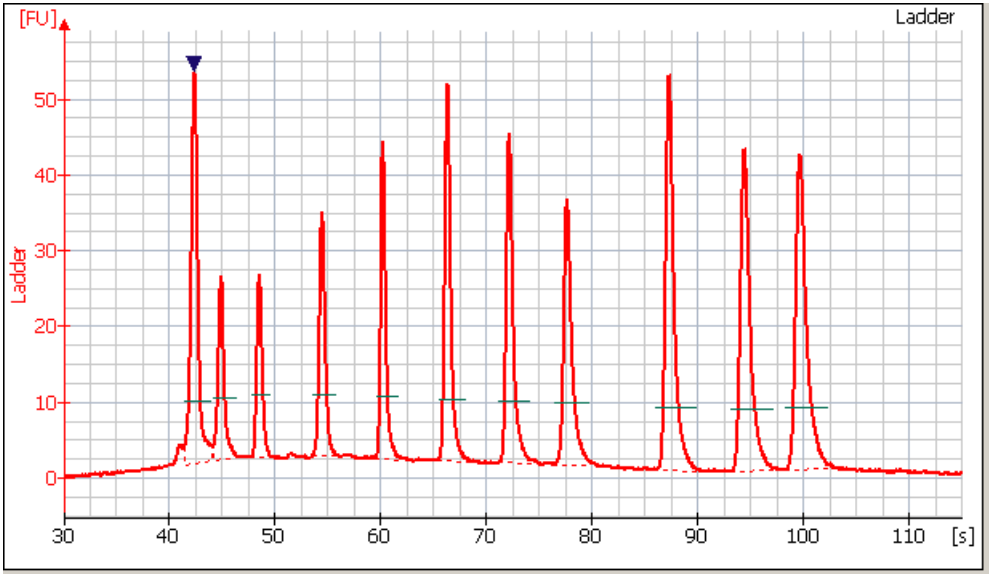
- "Changing the Data Analysis" on page 116
- "Manual Integration" on page 131
- "Reanalyzing a Chip Data File" on page 141
- "Comparing Samples from Different Electrophoretic Chip Runs" on page 143
- "How to Use the Form Mode" on page 159

Data Analysis: DNA

The data analysis process for DNA assays consists of the following steps:

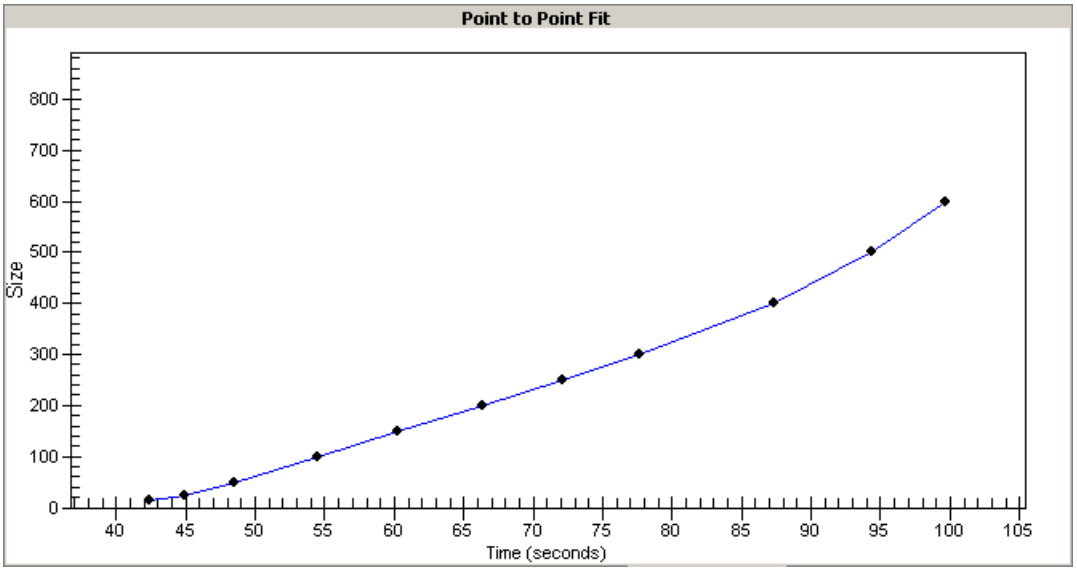
1. Raw data is read and stored by the system for all of the individual samples.
2. The data is filtered and the resulting electropherograms of all samples are plotted. You can change the settings of the data analysis after the run and reanalyze your data.
3. Peaks are identified for all samples and are tabulated by peak ID. You can change the settings of the peak find algorithm and reanalyze the data after the run has finished. (Note that peak find settings can be changed for all or only certain samples.)

4. A sizing ladder (see the following example electropherogram), which is a mixture of DNA fragments of known sizes, is run first from the ladder well. The concentrations and sizes of the individual base pairs are preset in the assay and cannot be changed.

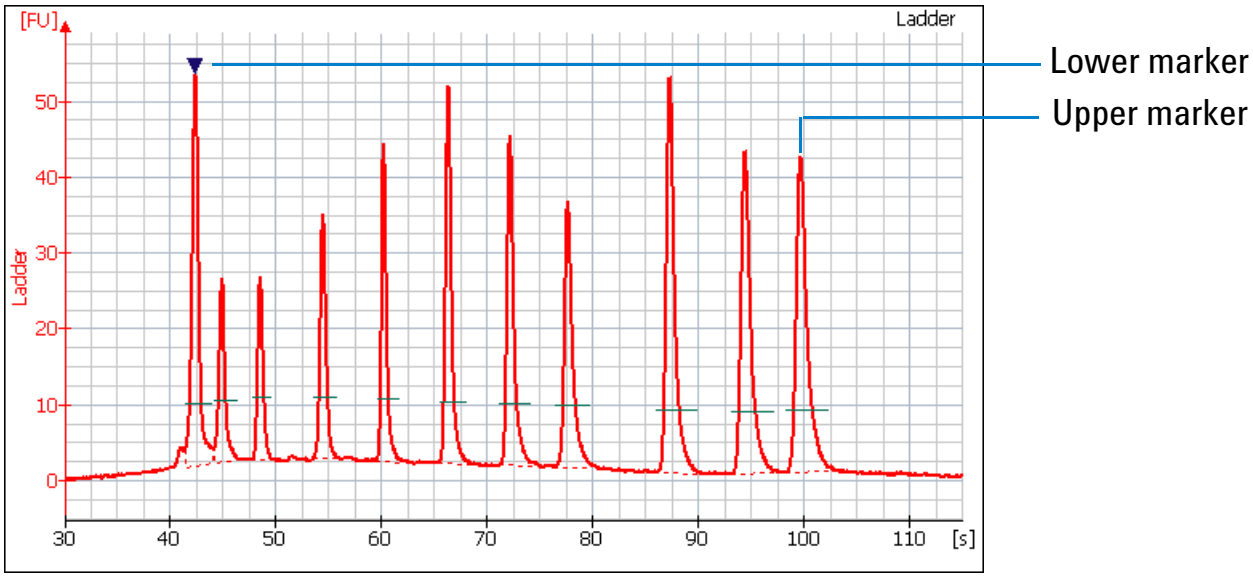


		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
▶		15	4.20	424.2	Lower Marker
2		25	2.00	121.2	Ladder Peak
3		50	2.00	60.6	Ladder Peak
4		100	2.00	30.3	Ladder Peak
5		150	2.00	20.2	Ladder Peak

5. A standard curve of migration time versus DNA size is plotted from the DNA sizing ladder by interpolation between the individual DNA fragment size/migration points. The standard curve derived from the data of the ladder well should resemble the one shown below.



6. Two DNA fragments are run with each of the samples, bracketing the DNA sizing range. The “lower marker” and “upper marker” are internal standards used to align the ladder data with data from the sample wells. The figure below shows an example of assigned marker peaks in a sample well.



NOTE

The software performs alignment by default. Turning automatic data analysis off suspends data analysis until you turn it on again.

7. The standard curve, in conjunction with the markers, is used to calculate DNA fragment sizes for each sample from the migration times measured.

8. To calculate the concentration of the individual DNA fragments in all sample wells, the upper marker, in conjunction with an assay-specific concentration against base-pair size calibration curve, is applied to the individual sample peaks in all sample wells.

NOTE

The software allows you to redefine the peaks chosen as upper and lower markers. A change in marker selection will cause quantitative changes in the calibration procedure, however, and therefore in the entire data evaluation.

---

9. If the check box *Rest. Digest* on the *Chip Summary Tab* is enabled, the 2100 expert software flags peaks that may have co-migrated:

		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
1	◀	15	4.20	424.2	Lower Marker
2		22	1.71	116.6	Possible Co-Migration of 4 Peaks
3		55	1.31	35.8	
4		104	4.22	61.3	Possible Co-Migration of 2 Peaks
5		141	3.19	34.4	
6		187	3.87	31.5	
7		235	4.74	30.6	
8		330	6.81	31.3	
9		381	7.99	31.8	
10		476	10.34	32.9	
11		512	9.31	27.6	
12	▶	1,500	2.10	2.1	Upper Marker

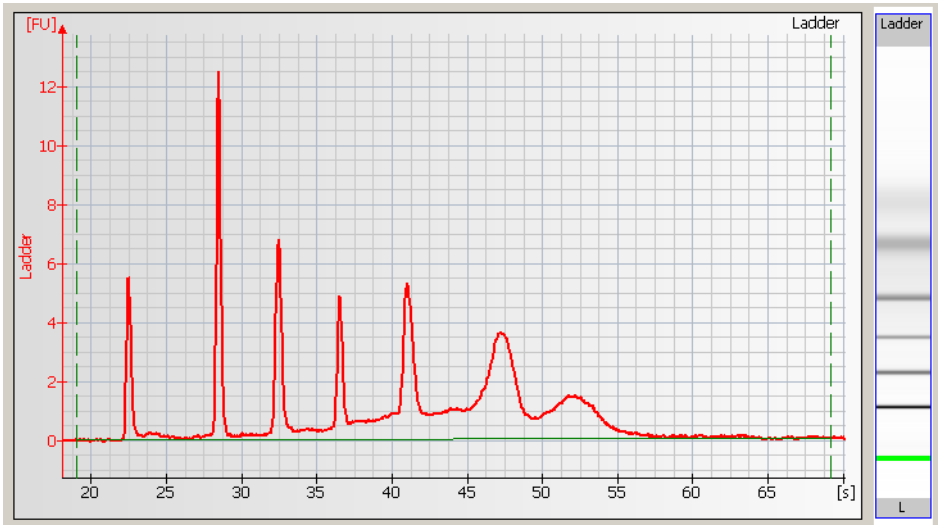
Results | Peak Table | Legend | Errors

Since it is assumed that the molarity of all the fragments in a restriction digest should be the same, any peaks or clusters having a molarity that is significantly larger than the rest are flagged as potentially co-migrating peaks, allowing you to examine them in more detail.

Data Analysis: RNA and Cy5-Labeled Nucleic Acids

The data analysis process for RNA and the Cy5-labeled nucleic acids assays consists of the following steps:

1. Raw data is read and stored by the system for all of the individual samples.
2. The data is filtered and the resulting electropherograms of all samples are plotted. You can change the settings of the data analysis after the run and reanalyze your data.
3. Fragments are identified for all samples and tabulated by peak ID. You can change the settings of the peak find algorithm for any or all samples and reanalyze the data.
4. An RNA ladder (containing a mixture of RNA of known concentration) is run first (see the electropherogram below). The concentrations and sizes of the individual base pairs are preset in the assay and cannot be changed.

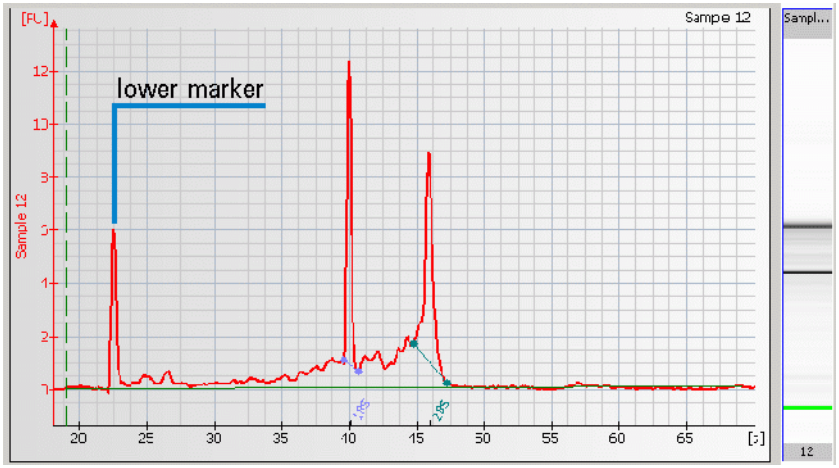


Electropherogram of RNA 6000 Ladder (Ambion, Inc. cat. no. 7152)

NOTE

Peak ratios for the RNA ladder may vary from one batch of RNA 6000 ladder to the next. Assay performance will not be affected by this variation.

5. For the Eukaryote or Prokaryote Total RNA assay, the RNA fragments (either 18S and 28S for eukaryotic RNA or 16S and 23S for prokaryotic RNA) are detected. After detection, the ratio of the fragment areas is calculated and displayed.



6. To calculate the concentration of the RNA, the area under the entire RNA electropherogram is determined. The ladder, which provides the concentration/area ratio, is applied to transform the area values into concentration values.

Alignment of RNA Samples

The marker solution that is part of each RNA LabChip kit, contains a 50 bp DNA fragment. This fragment is used as lower marker to align all samples.

By default the RNA alignment and the subtraction of the lower marker are enabled for RNA Nano assays.

The marker is displayed as the first peak in the electropherogram.

The RNA Integrity Number (RIN)

The RNA integrity number (RIN) is a tool designed to help scientists estimate the integrity of total RNA samples. The RIN extension automatically assigns an integrity number to a eukaryote total RNA sample analyzed on the Agilent 2100 bioanalyzer. Using this tool, sample integrity is no longer determined by the ratio of the ribosomal bands alone, but by the entire electrophoretic trace of the RNA sample, including the presence or absence of degradation products. In this way, interpretation of an electropherogram is facilitated, comparison of samples is enabled and repeatability of experiments is ensured.

Scope

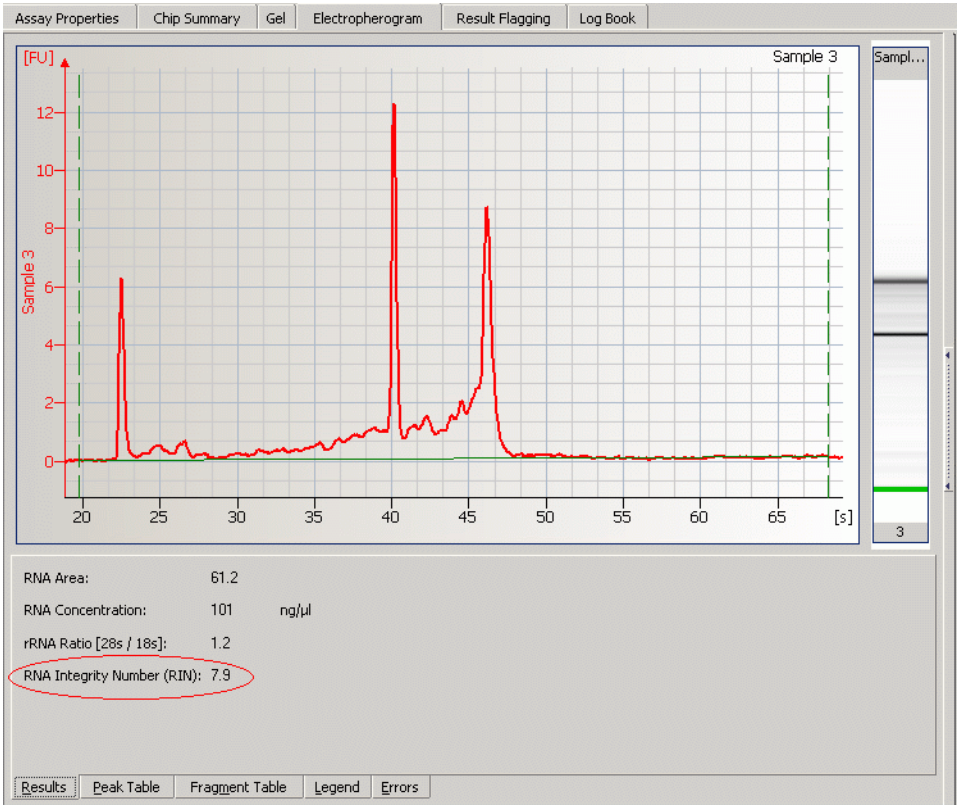
What the RIN can do:

- Obtain an assessment of the integrity of RNA.
- Directly compare RNA samples (e.g. before and after shipment, compare integrity of same tissue across different labs, etc.).
- Ensure repeatability of experiments (e.g. if RIN shows a given value and is suitable for microarray experiments, then the RIN of the same value can *always* be used for microarray experiments given that the same organism/tissue/extraction method was used).

What it *cannot* do:

- Tell a scientist ahead of time whether an experiment will work or not if no prior verification was done (e.g. RIN of 5 might not work for microarray experiments, but might work well for an appropriate RT-PCR experiment. Also, an RIN that might be good for a 3' amplification might not work for a 5' amplification).

The computation of the RIN is part of data analysis for total RNA samples. The computed RNA integrity number is shown on the *Results* sub-tab of the *Gel* or *Electropherogram* tab of the *Data* context. It is also included in XML export files and in printed reports.



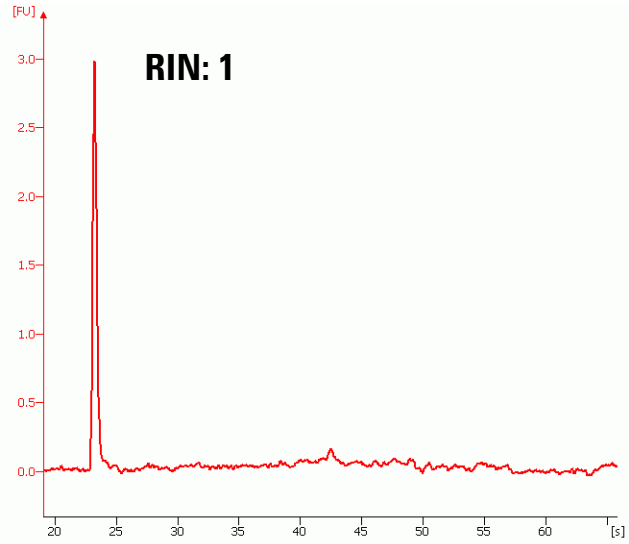
NOTE

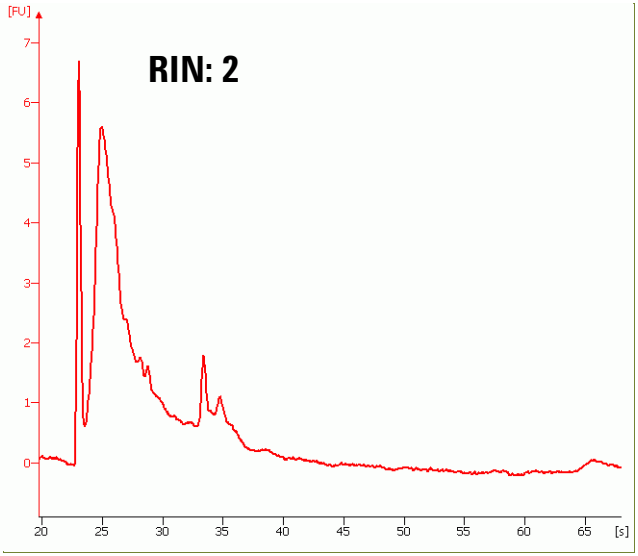
Until now, the computation of the RIN has only been validated for eukaryote total RNA Nano samples. The 2100 expert software also calculates the RIN for prokaryote total RNA samples and for the RNA 6000 Pico assay. Be aware that for these samples, the RIN has not been validated in extensive downstream experiments.

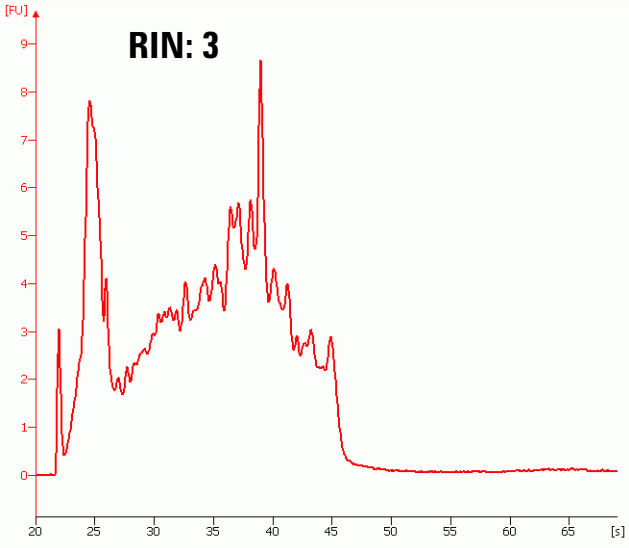
Although the lower quantitative limit of the RNA 6000 Nano assay is specified as 25 ng/µl it is recommended to use at least 50 ng/µl for a meaningful RNA integrity number. When using lower concentrations, higher sample to sample variances of the RIN may be observed.

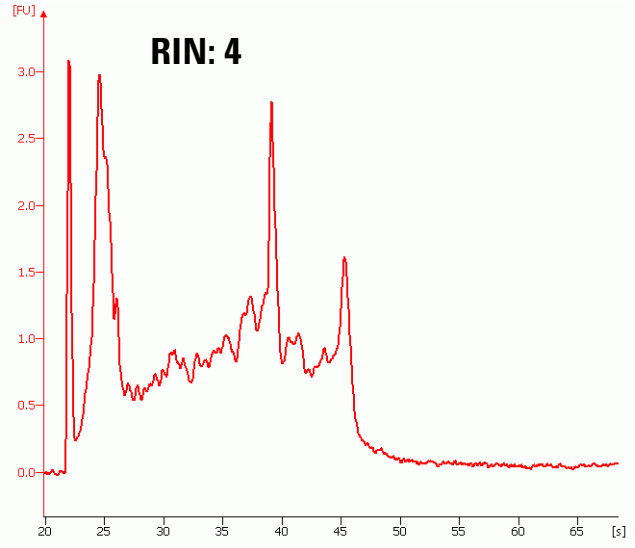
Examples for RNA Integrity Numbers

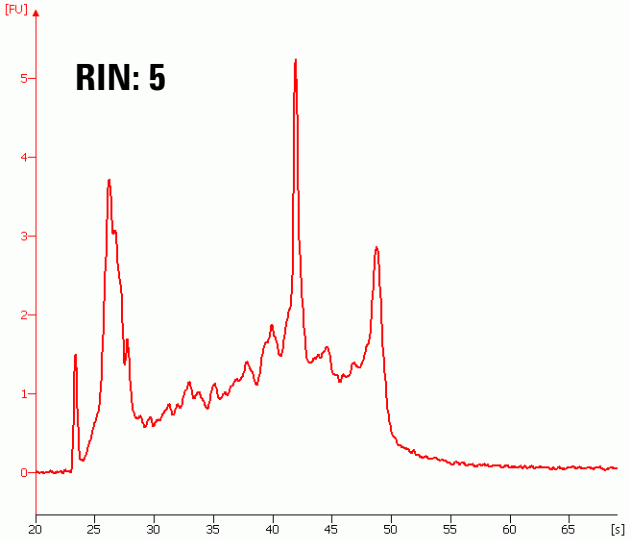
A database of about 1300 mammalian total RNA samples was created using the RNA 6000 Nano assay. The samples came from different species (mainly human, rat and mouse), tissues, preparation methods, concentrations and degradation states. All samples were classified according to their degradation state. Numbers from ’1’ to ’10’ were used as labels. ’10’ stands for a perfect RNA sample without any degradation products, whereas ’1’ marks a completely degraded sample. The labels in-between are used to indicate progressing degradation states of the RNA sample. The following figure shows typical representatives for each of the 10 RNA integrity classes.

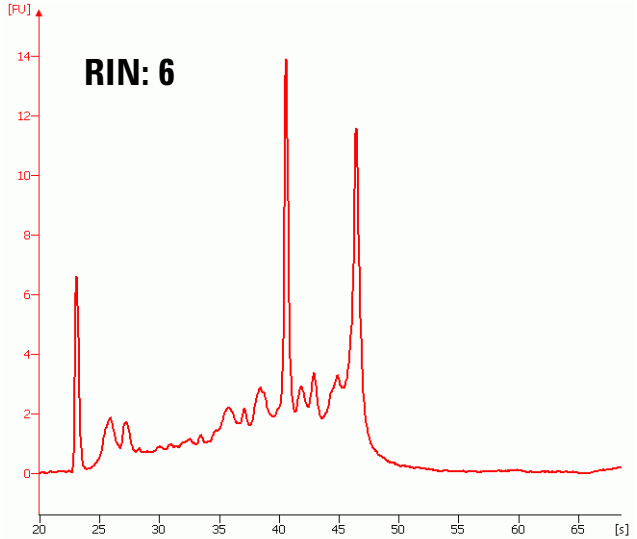

RIN: 1
[FU]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
20
25
30
35
40
45
50
55
60
[s]

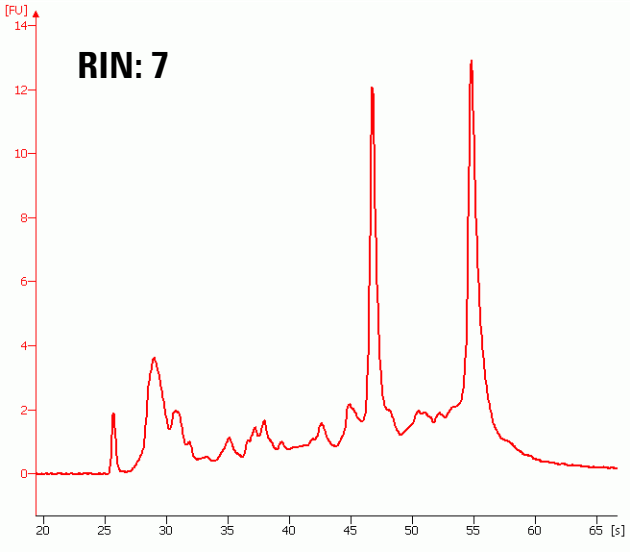

RIN: 2
[FU]
7
6
5
4
3
2
1
0
20
25
30
35
40
45
50
55
60
65
[s]

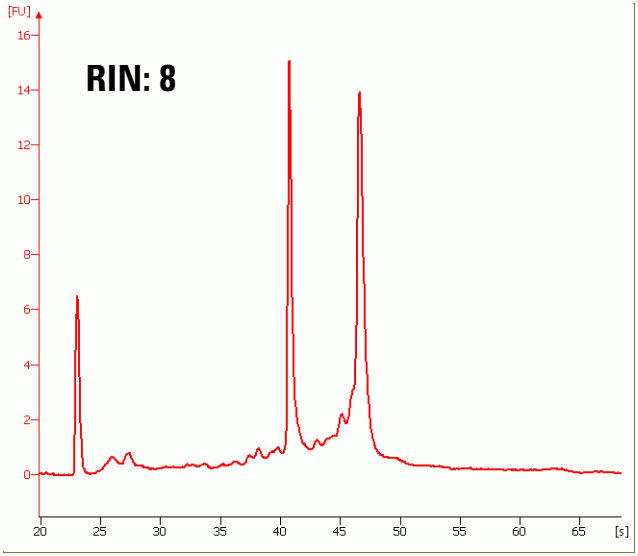

RIN: 3
[FU]
9
8
7
6
5
4
3
2
1
0
20
25
30
35
40
45
50
55
60
65
[s]

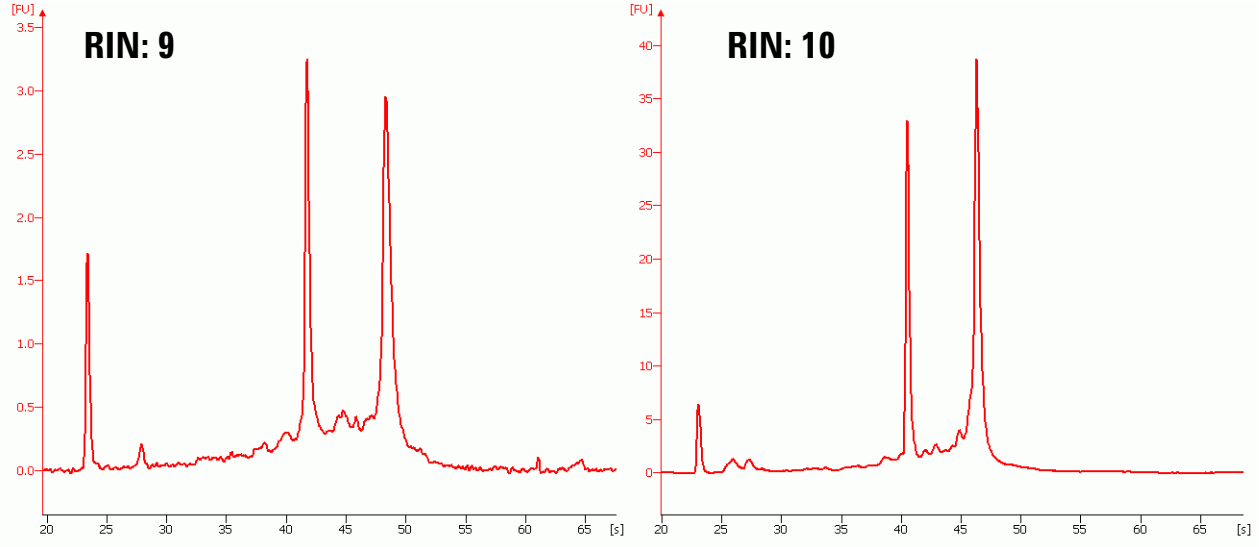

RIN: 4
[FU]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
20
25
30
35
40
45
50
55
60
65
[s]


[FU]
RIN: 5
[s]


[FU]
RIN: 6
[s]

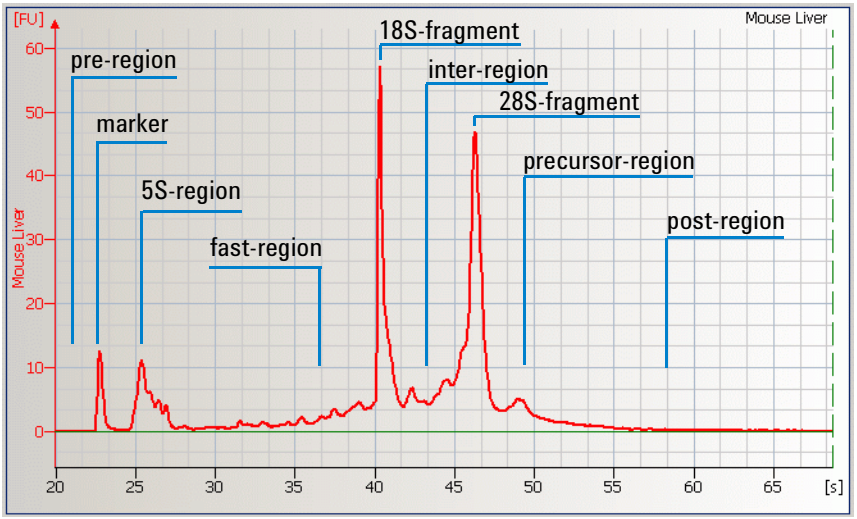

[FU]
RIN: 7
[s]


[FU]
RIN: 8
[s]


RIN: 9
[FU]
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
20
25
30
35
40
45
50
55
60
65
[s]
RIN: 10
[FU]
40
35
30
25
20
15
10
5
0
20
25
30
35
40
45
50
55
60
65
[s]

Computation of the RNA Integrity Number and Signal Anomalies

For the computation or the RNA integrity number, the electropherogram is partitioned into regions as shown in the figure below. The lower marker and the 18S and 28S fragments divide the electropherogram into nine regions:



Signal Anomalies

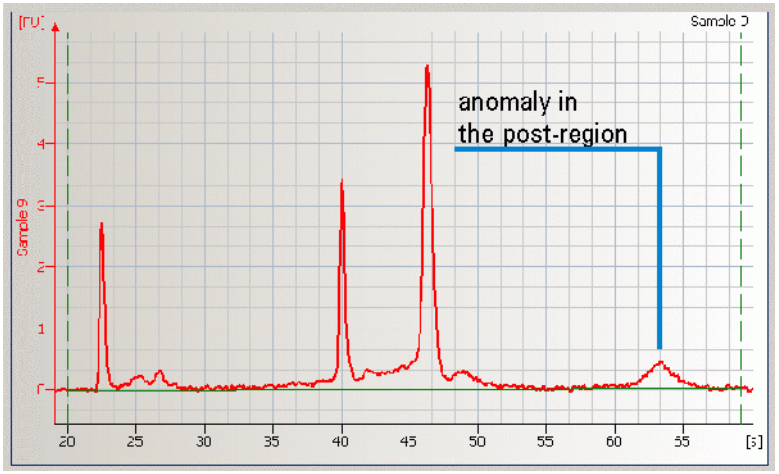
In addition to the computation of the RIN, the data analysis detects various unexpected signals, disturbing the computation of the RIN. Such disturbances are called anomalies. Region anomaly detectors recognize unexpected signals in each region. If detected, the anomaly is displayed in the *Error* sub-tab of the *Electropherogram* and *Gel* tab.

Anomaly Description	Critical?
Unexpected baseline signal	Yes
Unexpected signal in pre-region	No
Unexpected signal in 5S-region	Yes
Unexpected signal in fast-region	Yes
Unexpected signal in inter-region	Yes
Unexpected signal in precursor-region	No
Unexpected signal in post-region	No
Unexpected ribosomal ratio	Yes
Unexpected sample type	Yes
Unexpected lower marker (compared to previous well)	No

Two categories of anomalies were introduced, critical and non-critical. Anomalies in regions interfering with the customer sample RNA are considered critical. The corresponding gel lane is flagged red.

The baseline anomaly, for example, is detected for signals with fluctuating or steep baseline. The ribosomal ratio anomaly detects unexpected ratios of the 28S fragment area and the 18S fragment area. The unexpected sample type anomaly is detected for samples which do not fit the standard total RNA profile.

If a non-critical anomaly is detected, the RIN can still be computed accurately. Therefore non-critical anomalies are not flagged. Non-critical region anomalies are pre-region anomaly, precursor-region anomaly and post-region anomaly. The electropherogram below gives an example for a non-critical anomaly in the post-region.

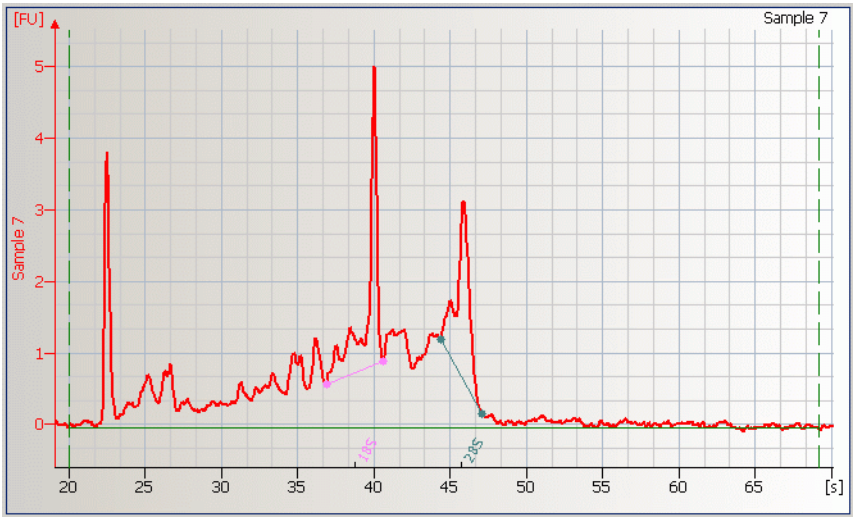


Troubleshooting the RIN

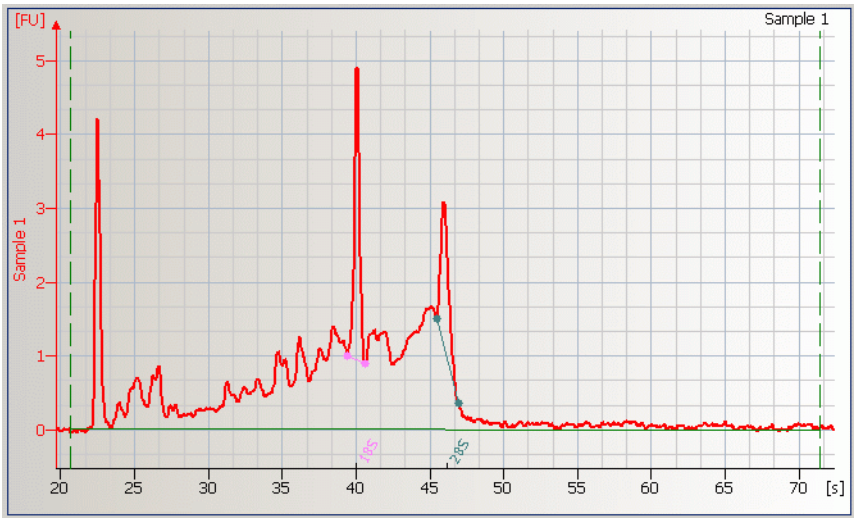
To obtain meaninful and reproducible results, the lower marker and ribosomal bands must be identified correctly. Although the ribosomal fragment identification has been improved, in rare cases (i.e. when analyzing degraded RNA samples) the fragment baseline is not properly set. In this case the user should adjust the baseline settings manually.

Example:

'Incorrect' software identification of the ribosomal fragments: RIN=7.2



RNA integrity number after the manual adjustment: RIN=5.7

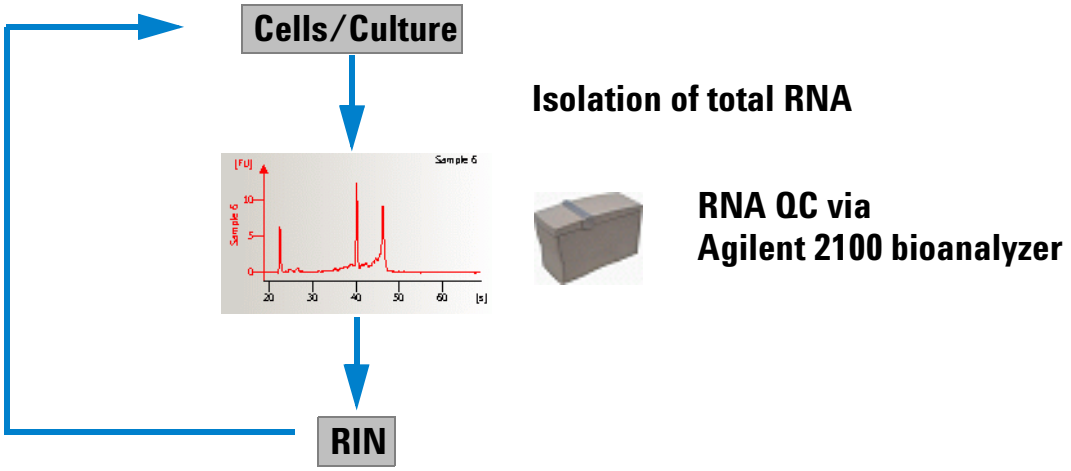


On details on how to adjust the lower marker and ribosomal bands, please refer to "Changing the Data Analysis" on page 116.

Use Models for the RNA Integrity Number

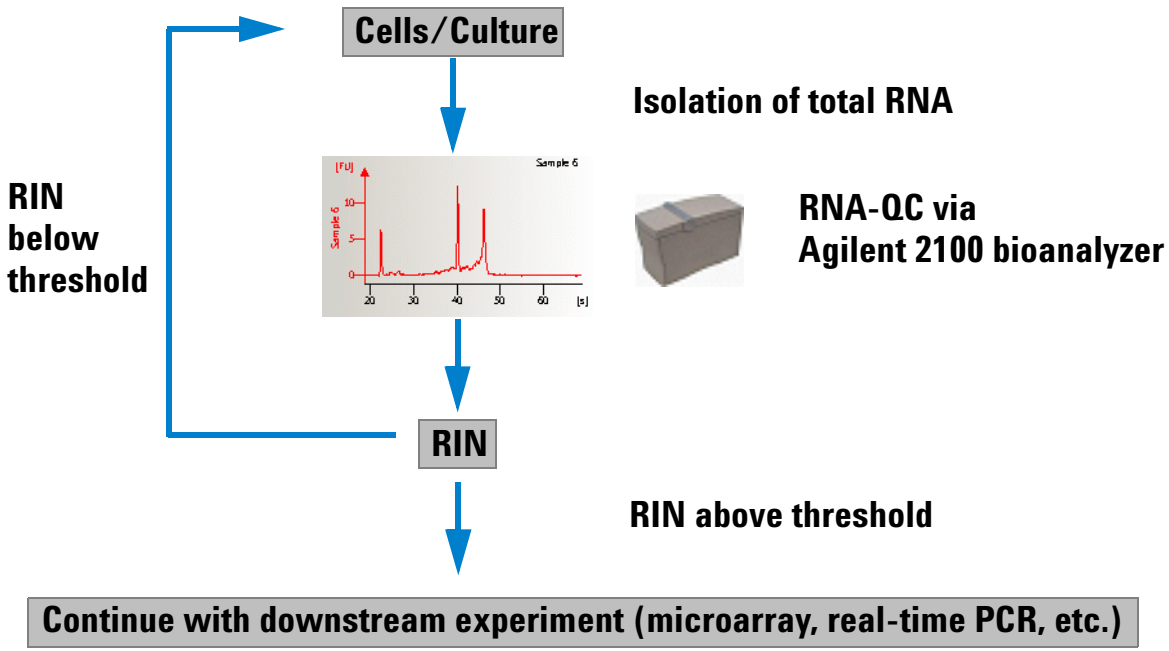
To take full advantage of the RIN feature, a 2 step use-model is suggested:

1 Determine the threshold value for the RIN that results in meaningful downstream experiments:



Correlate RIN with downstream experiment and determine threshold RIN for meaningful results (iterative process)

2 Run standard experiment and use RIN to determine if sample integrity is sufficient:



RNA Integrity Number Setpoints

Various setpoints are available to customize the display of the RIN (RNA Integrity Number). With these setpoints, you can modify the predefined thresholds for anomaly detection. You can find them in the advanced user mode of the setpoint explorer.

To adjust the setpoints for a single sample, switch to the *Local* tab of the setpoint explorer and open the *RNA Integrity Number* group.

To adjust the setpoints for the whole chip, switch to the *Global* tab of the setpoint explorer and open the *RNA Integrity Number* group in the *Sample Setpoints* group. For the chip, you can additionally switch between integer and decimal representation of the RIN.

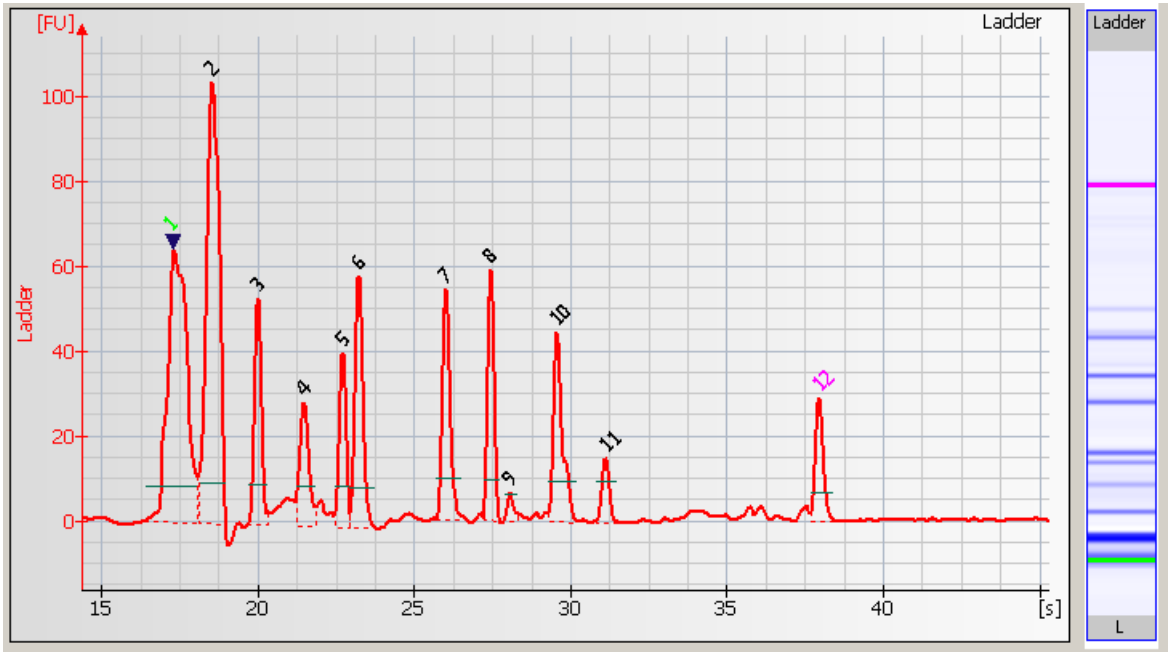
For more information on how to use the setpoint explorer, see "About the Setpoint Explorer" on page 117.

Data Analysis: Protein

The data analysis process for protein assays consists of the following steps:

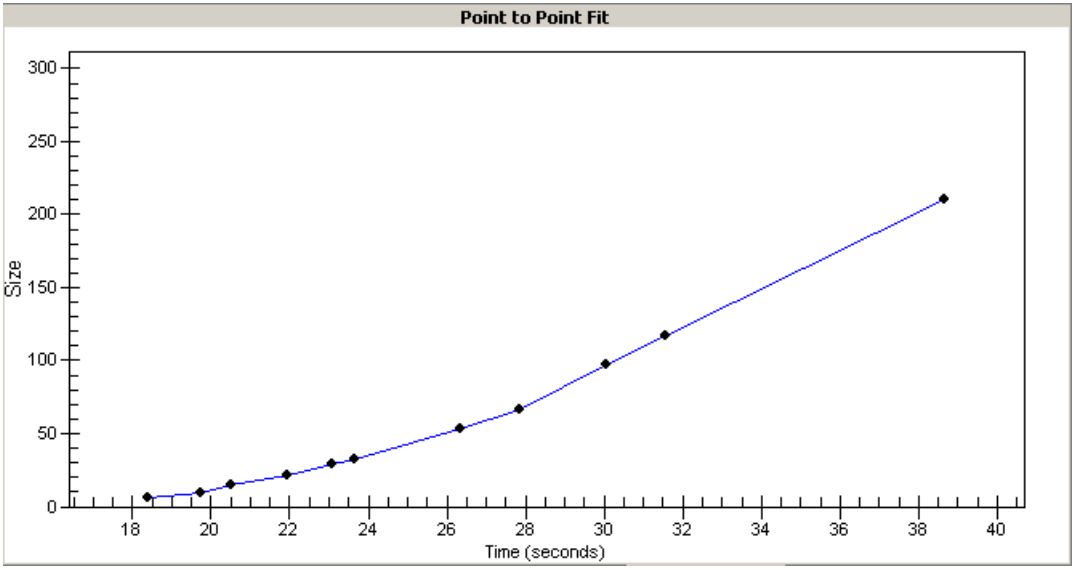
1. Raw data is read and stored by the system for all of the individual samples.
2. The data is filtered and the resulting electropherograms of all samples are plotted. You can change the settings of the data analysis after the run and reanalyze your data.
3. Peaks are identified for all samples and are tabulated by peak ID. You can change the settings of the peak find algorithm and reanalyze the data after the run has finished. (Note that peak find settings can be changed for all or only certain samples.)

4. A sizing ladder (see the example electropherogram below), which is a mixture of proteins of different known sizes, is run first from the ladder well. The sizes of the individual proteins are preset as kDa in the assay and cannot be changed. Please note that the concentrations may vary slightly from ladder lot to ladder lot.

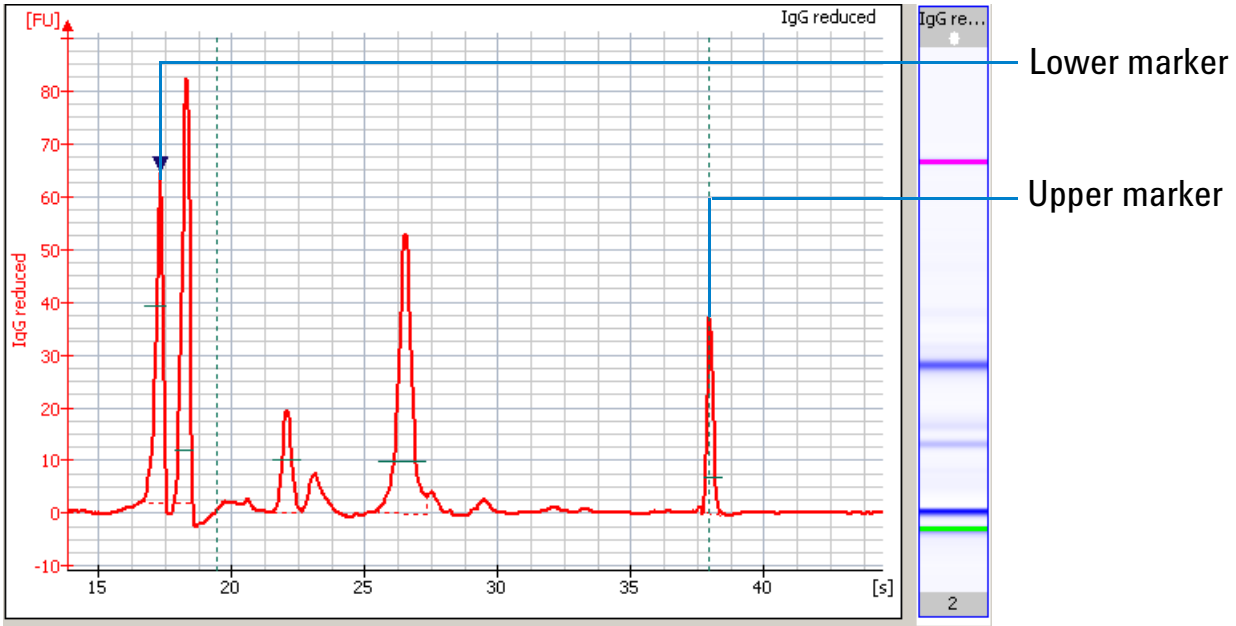


		Size [kDa]	Observations
▶	◀	6.0	Lower Marker
2		9.0	Ladder Peak
3		14.4	Ladder Peak
4		21.5	Ladder Peak
5		29.0	Ladder Peak
6		32.5	Ladder Peak
7		53.0	Ladder Peak
8		66.7	Ladder Peak

5. A standard curve of migration time versus size is plotted from the sizing ladder by interpolation between the individual protein size/migration points. The standard curve derived from the data of the ladder well should resemble the one shown below.



6. Two proteins are run with each of the samples, bracketing the sizing range. The “lower marker” and “upper marker” proteins are internal standards used to align the ladder data with data from the sample wells. The figure below shows an example of assigned marker peaks in a sample well.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations	Area
1	◀	6.0	0.0	0.0	0.0	Lower Marker	19.9
2		8.5	0.0	0.0	0.0	System Peak	27.5
3		25.3	140.7	0.0	23.7		8.5
4		58.1	451.7	0.0	76.3		32.6
5	▶	210.0	100.0	0.0	0.0	Upper Marker	10.3

NOTE

The software performs alignment by default. Turning automatic data analysis off suspends analysis until you turn it on again.

7. The standard curve, in conjunction with the markers, is used to calculate protein sizes for each sample from the migration times measured.

8. To calculate the concentration of the individual proteins in all sample wells, the upper marker is applied to the individual sample peaks in all sample wells.

NOTE

The software allows you to define upper and lower markers yourself. A change in the selection of the markers will lead to quantitative changes in the calibration procedure, however, and therefore in the entire data evaluation.

Protein Absolute Quantitation

Absolute quantitation is calculated based on the relative concentration of a sample and the user-defined standard and the known concentration of this user-defined standard.

For protein samples you can enable the use of calibration for each sample and enter the concentration of the standard protein. This allows you to generate a calibration curve, which can be used to analyze and quantitate this protein within different samples on the same chip.

Using Calibration in Protein Assays

The calibration feature for protein assays allows absolute quantitation based on external standard calibration.

On the *Chip Summary* tab, use the sample table on the *Sample Information* sub-tab to define the samples that you want to use as calibration standards and enter a concentration.

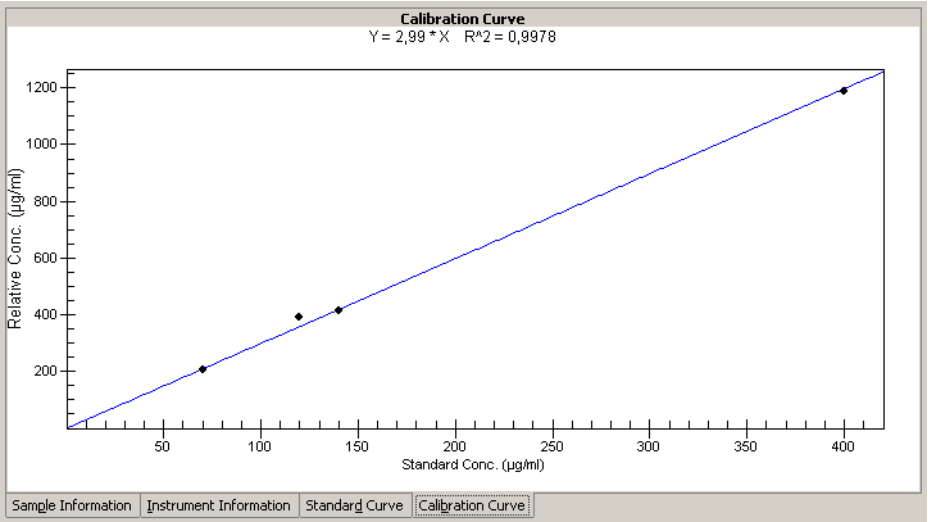
	Sample Name	Sample Comment	Use For Calibration	Conc.[µg/ml]	Status
1	beta LG		☐	0	✓
2	beta LG		☑	70	✓
3	beta LG		☑	140	✓
▶	beta LG		☑	400	✓
5	ovalbumin NR	non-reduced	☐	0	✓
6	ovalbumin R	reduced	☐	0	✓
7	insulin B chain	20 ug/ml	☐	0	✓
8	standard A		☐	0	✓
9	beta LG	500 ug/ml	☑	120	✓
10	standard B		☐	0	✓

The calibration standard should be run in different concentrations to generate a calibration curve. The software will automatically produce this calibration curve based on these inputs to determine the actual concentration of samples within the same chip. In the peak tables of the samples, a remark is added to the observation column to identify the calibration protein and the calibrated proteins:

		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		3,5	0,0	0,0	0,0	Lower Marker
2		4,2	0,0	0,0	0,0	System Peak
3		4,7	0,0	0,0	0,0	System Peak
4		16,5	31,4	0,0	6,7	
▶		18,5	392,6	131,2	83,7	Calibration Protein
6		28,9	45,1	0,0	9,6	
7		53,0	100,0	0,0	0,0	Upper Marker

		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		3,5	0,0	0,0	0,0	Lower Marker
2		4,1	0,0	0,0	0,0	System Peak
3		4,5	0,0	0,0	0,0	System Peak
▶		18,4	22,1	7,4	43,2	Calibrated Protein
5		29,1	29,1	0,0	56,8	
6		53,0	100,0	0,0	0,0	Upper Marker

The calibration curve can be displayed by switching to the *Calibration Curve* sub-tab on the *Chip Summary* tab.



Smear Analysis

The 2100 expert software allows to perform a smear analysis for all electrophoresis assays.

When the smear analysis is enabled, the software allows you to define regions of interest. These regions are used to define the area of broad peaks and determine their part of the total area. Smear analysis provide a means to analyze broad signals that can be hardly evaluated with the normal peak assignment.

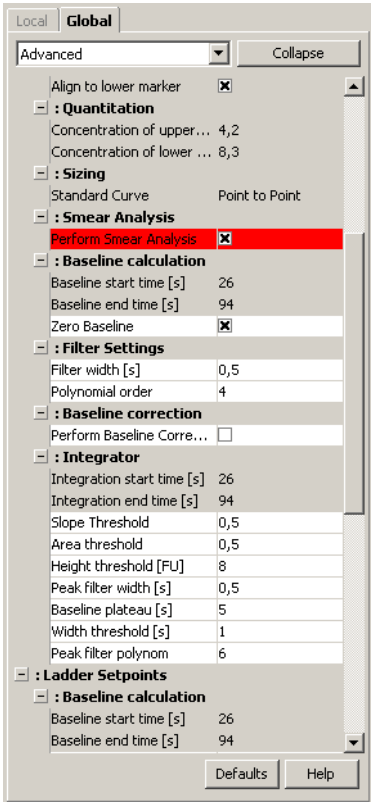
You therefore can define regions of interest that contain the peaks (base pair size) that you are interested in. For these regions you can determine the covered area in relation to the total area.

Enabling Smear Analysis

To enable smear analysis:

1. Go to the *Electropherogram* tab in the *Data* context:
2. Go to the setpoint explorer and select the *Local* or *Global* tab, depending on which samples should be analyzed.
3. Select the *Advanced* mode.

4 Under *Smear Analysis*, select the check box *Perform Smear Analysis*.



The *Region Table* sub-tab is added to the *Electropherogram* tab.

Performing Smear Analysis

After enabling the smear analysis in the setpoint explorer, you are able to insert regions of interest in the electropherogram.

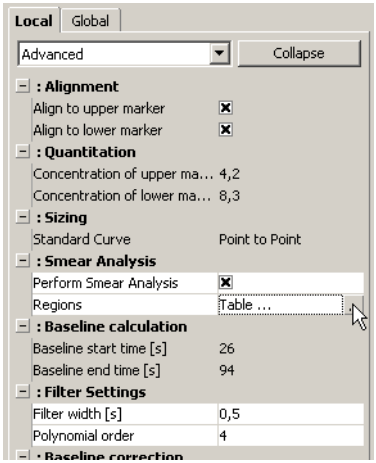
To do so:

1. Select the *Region Table* sub-tab in the *Electropherogram* tab.
2. Right-click the electropherogram and select *Add region*.

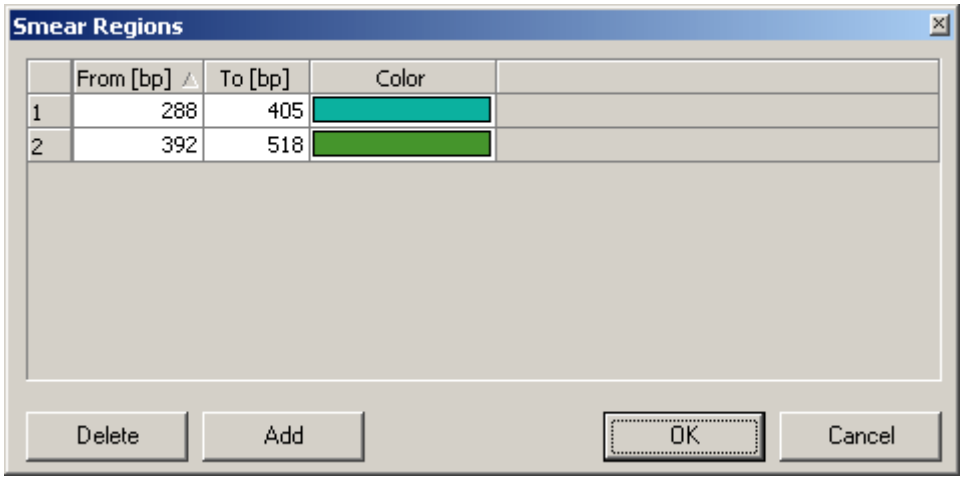
   A region will be inserted into the electropherogram. The *Region Table* shows the values for the inserted region.
3. Repeat the previous step until the number of required regions is inserted.
4. Adjust the regions by directly moving the dashed lines in the electropherogram.
5. To remove a region, right-click the dashed line in the electropherogram and select *Remove Region* from the context menu.

NOTE

The smear analysis table can be directly edited by selecting the region table under *Smear Analysis* in the setpoint explorer.



In the smear analysis table, you can edit the *Region Start Size* and *Region End Size*, for example:



Changing the Data Analysis

Different sets of parameters (data analysis setpoints) can be changed in the software in order to modify the data evaluation for sample analysis:

- Filtering parameters
- Peak find parameters for all samples/peak height for individual samples
- Enabling smear analysis
- Align to upper and/or lower marker
- Adding/deleting ribosomal fragments (for RNA assays only)
- Manual integration (for protein and DNA assays only)
- Absolute quantitation (for protein assays only)

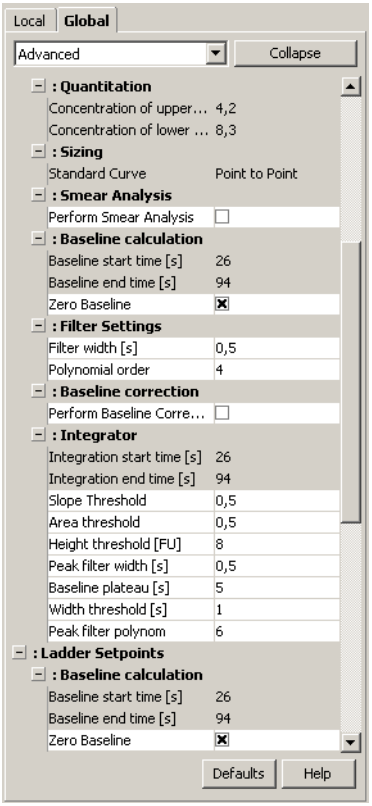
These settings can be made before a new run is started or when reanalyzing a previously saved data file.

About the Setpoint Explorer

The tool allowing you to modify the data analysis setpoints is the *Setpoint Explorer*. The setpoint explorer is accessible from:

- *Assay Properties Tab*
- *Electropherogram Tab (Single/Grid View)*
- *Gel Tab*

On the *Assay Properties* tab, the setpoint explorer is always visible and lets you modify setpoints *globally* (for all samples):



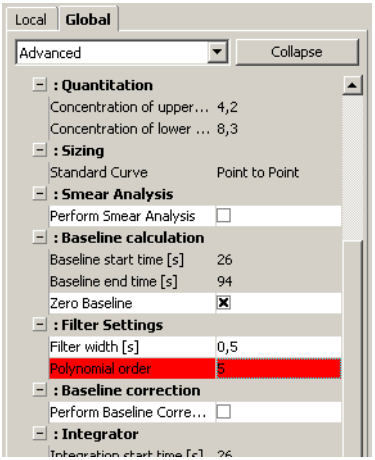
To show the setpoint explorer, on the *Electropherogram/Gel* tab, click the vertical bar on the right edge of the application window:

The setpoint explorer appears.

For electrophoretic assays, you can modify the setpoints

- *globally*, that is, for all samples (*Global* tab)
- *locally*, for the current sample (*Local* tab)

Click the + nodes to expand, and the – nodes to collapse branches. Setpoints that you can change are white. To edit a setpoint, double-click the value, enter the new value, and press enter. They are applied automatically.



When you try to change any global setpoints where local settings have been applied, the software prompts you as to whether you want to overwrite the local (custom) settings.

If you decide to overwrite the custom sample settings, all local settings you made will be discarded. If you decide not to overwrite the custom sample settings, the global settings will not be applied where local settings have been changed.

Filtering Setpoints

The first step the software takes in analyzing the raw data is to apply data filtering. The following filtering setpoints can be changed:

Filter Width	Defines the data window, given in seconds, used for averaging. The broader the filter width, the more raw data points are used for averaging. As a result, the noise level will decrease, but peaks will become lower and broader. Overall, changing the Filter Width has more effect on the result of the filtering procedure applied then does changing the Polynomial Order.
Polynomial Order	This setting is used to define the power series applied to fit the raw data. The higher the number, the more the fit function will follow the noisy raw data curve. As a result, the noise level of the filtered curve will increase.

Integrator Setpoints

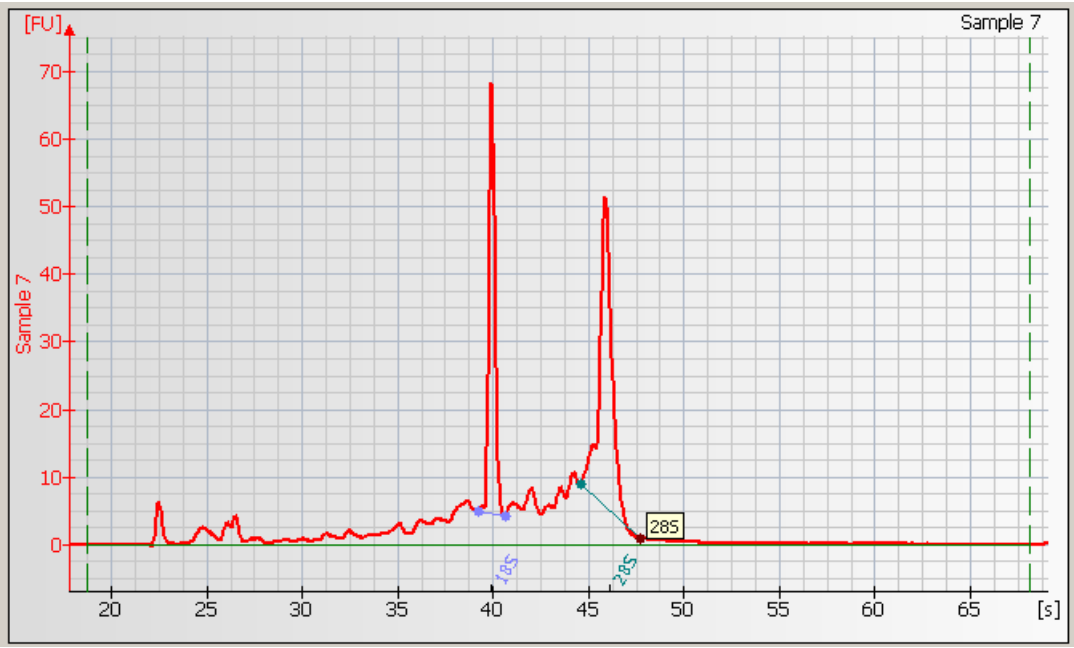
After data filtering, the peak find algorithm locates the peaks and calculates the local peak baselines. The algorithm begins by finding all the peaks above the noise threshold in order to determine the baseline, after which any peaks below the noise threshold are rejected. A local baseline is calculated for each peak to allow for baseline drift.

The four integrator setpoints that can be changed are:

Slope Threshold	The *Slope Threshold* setpoint determines the difference in the slope that must occur in order for a peak to begin. The inverse of this value is used to determine the peak end.
Area Threshold	The *Area Threshold* determines the minimum amount of peak area that must be detected before a peak is recognized.
Height Threshold	The *Height Threshold* setpoint determines whether a peak is kept. It represents the minimal peak height. For each peak, the difference between the start point value and the center point value (local baseline) must be greater than the *Height Threshold* value.
Peak Filter Width	The *Peak Filter Width* setpoint determines the minimum amount of time that must elapse before a peak is recognized.
Baseline Plateau	The *Baseline Plateau* setpoint is a parameter that assists in finding peaks. The signal is recognized to be at baseline whenever the slope of the data is less than the *Slope Threshold* setpoint (either positive or negative) for longer than the time set for the Baseline Plateau. This setting rejects brief, low slope areas such as between non-baseline-resolved peaks.

Manually Moving Fragment Start and End Points (RNA and Cy5-Labeled Nucleic Acids)

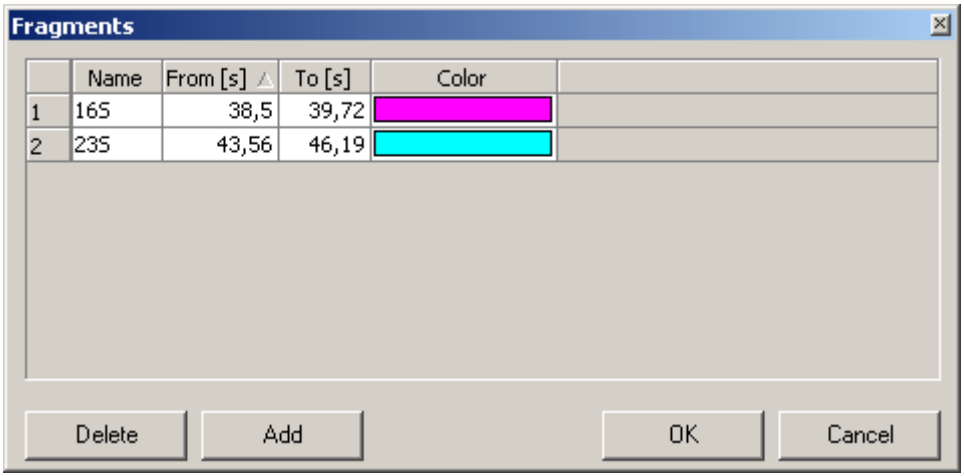
It is also possible to alter the start and end points manually for individual fragments in an RNA or Cy5-labeled nucleic acids assay. The integration borders of detected RNA-fragments are displayed in the *Fragment Table* sub-tab. Zooming in on the base of a particular fragment allows you to see the start and end points. Placing the cursor over one of these points changes the cursor to a pointing hand, allowing you to click and drag the point along the line of the fragment until it is positioned as desired.



Move any other start or end points as desired.

TIP

The fragment table can be directly edited in the setpoint explorer:



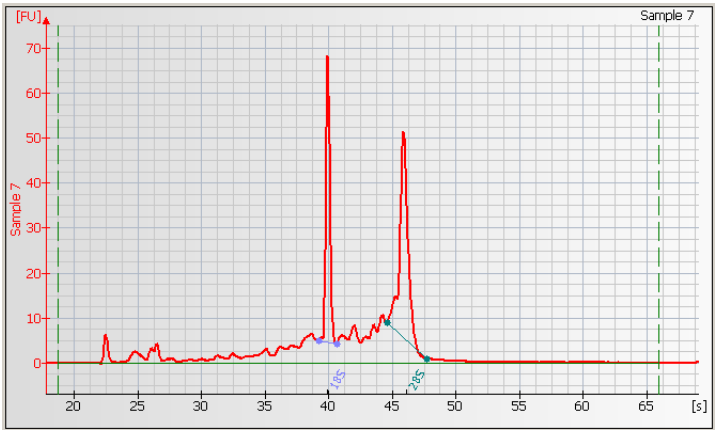
NOTE

Changing the start or end points of the fragment will change the calculated rRNA ratio. It might be convenient to pause the automatic analysis (*Electropherogram > Pause Automatic Analysis*) until all changes are done.

Setting the Baseline for Calculation of RNA Concentration

At low signal-to-noise ratios, the baseline that defines the area used for calculating the concentration of RNA assays is highly dependent on the settings for the Start and End Time. You can adjust the Start and End Times manually (thereby adjusting the baseline)

to ensure a good result even at very low signal-to-noise ratios. Choose a single sample. Two vertical green long-dashed lines indicating the setpoints for the Start and End Times (with the baseline drawn between them) are displayed in the window.



Move the cursor over the long-dashed line on the left (Start Time setting) and drag the line to the desired position. Do the same with the long-dashed line on the right (End Time setting) until you have a flat baseline.

NOTE

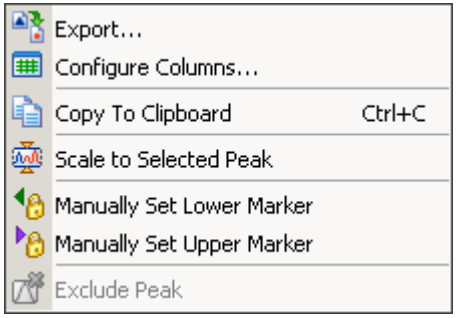
Changing the start and end times will change the calculated RNA concentration. It might be convenient to pause the automatic analysis (*Electropherogram > Pause Automatic Analysis*) until all changes are done.

Assigning Upper and Lower Marker Peaks

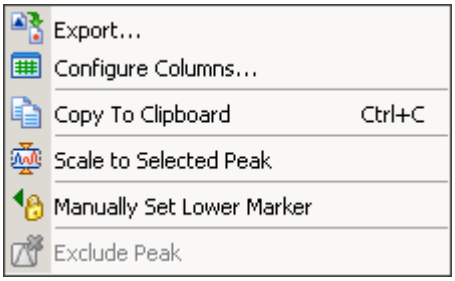
For each DNA or protein sample, the upper and lower marker peaks are assigned first and then the data is aligned so that the sample markers match the ladder markers in time, allowing the size and concentration of the sample peaks to be determined. RNA samples are aligned to a lower marker exclusively.

The first peak is assigned to be the lower marker and is then offset to match the lower marker in the ladder. The upper marker is then assigned to the last peak in the sample well or to the peak nearest the ladder's upper marker. See an example of assigned marker peaks below.

If you get unexpected peaks in the ladder analysis or find that the markers have been set incorrectly, you may exclude peaks manually from the ladder or set a peak to be used as a marker. Right-clicking in the peak table causes a context menu to appear, allowing you to do so:



In case the 2100 expert software did not detect the lower marker in RNA samples correctly, you are able to manually assign it in the same way.



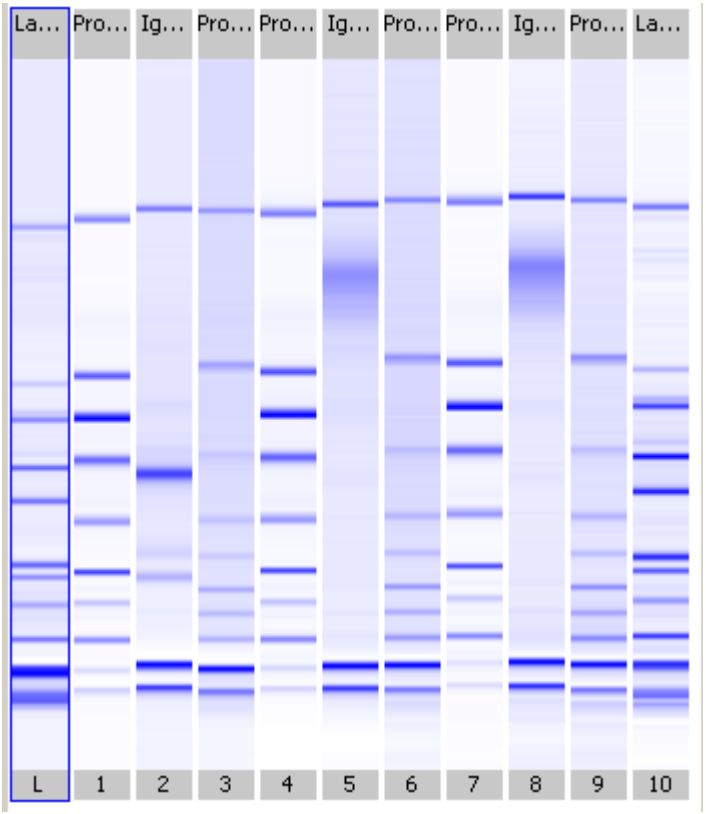
CAUTION

Excluding a peak or manually setting a peak to be an upper or lower marker may cause errors during analysis.

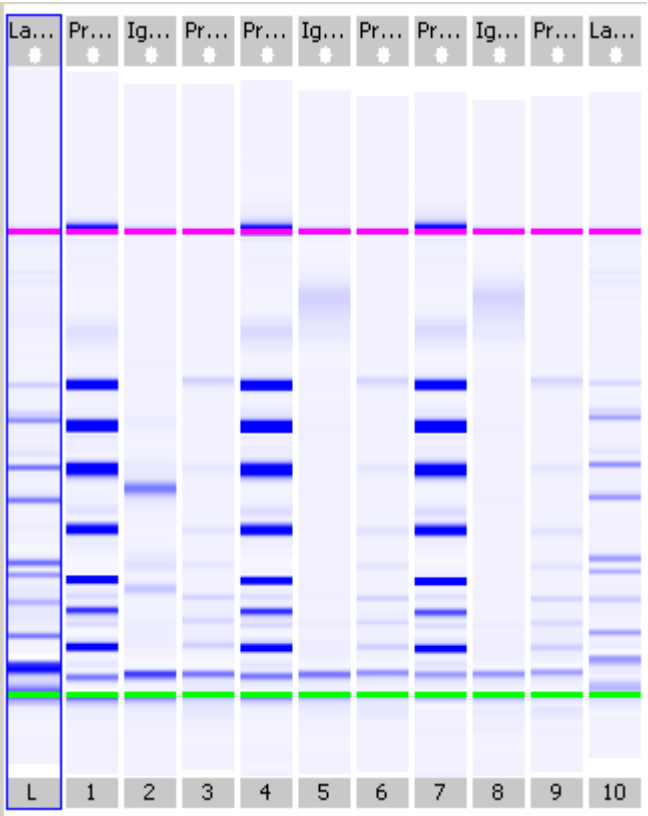
Aligning or Unaligning the Marker Peaks

The upper and lower are then *aligned* to the ladder markers by resampling the sample data in a linear stretch or compression using a point-to-point fit.

Data before alignment:



Markers aligned to the ladder:



If the sample marker peaks are either more than twice as far apart or less than half as far apart as the ladder markers, they are assumed to be the wrong peaks, and analysis of the sample stops, producing the error “Marker peaks not detected”.

NOTE

With DNA and protein assays, the height of marker peaks is assay dependent. Ladder peaks are analyzed to calculate a marker peak threshold that is used to locate the marker peaks in the sample wells. If the marker peaks found using this calculated method fail to align with those of a sample, the 2100 expert software will use the minimum peak height threshold setting instead (if this value is lower than the value for the marker peak). For example, the calculated threshold might be too high to find the sample's markers if they happen to be very small for some reason. Either no markers will be found or the wrong peaks will be assumed to be markers and these may not align with the ladder markers. Consequently, the software attempts to use the minimum peak height threshold that, if it is set low enough, will catch the real markers, allowing the sample to align.

NOTE

After alignment, peaks are shown with relative migration times that are different from the real times with data unaligned.

Manual Integration

For DNA and Protein assays, the 2100 expert software allows to manually integrate peaks. Manual integration allows you to move, add or delete peak baselines.

TIP

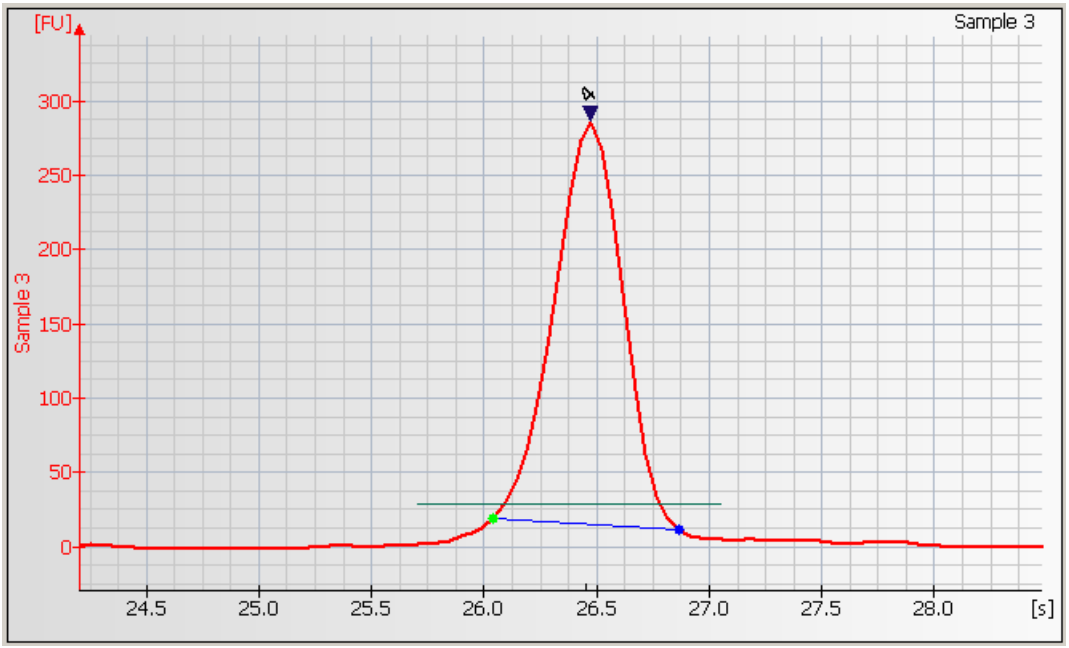
To move a peak baseline, point along the vertical line, press the CTRL key and left mouse button. To move a peak baseline, point along the signal, press the left mouse button only.

Example: Adjusting peak baselines

To manually change peak baselines:

1. Switch to the *Electropherogram* tab in the *Data* context and zoom into the electropherogram to enlarge the peak of interest.
2. Select *Electropherogram* > *Manual Integration* to switch off the automatic integration. As an alternative you can click the *Manual Integration* button in the toolbar.

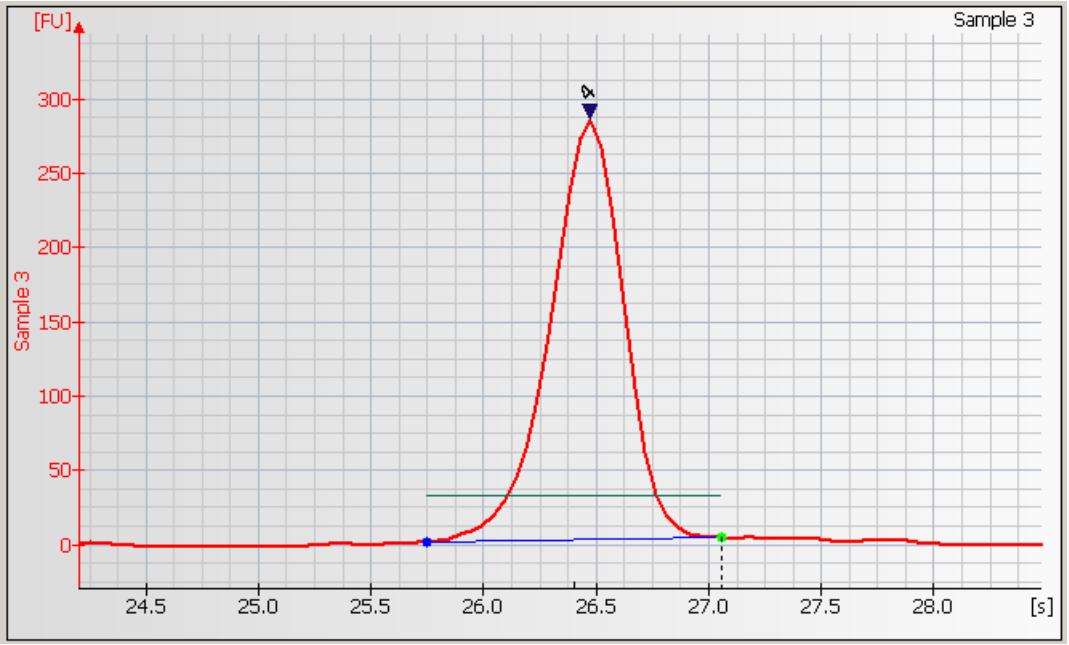
The baseline points become visible as blue or green dots. Highlighted baseline points are labelled green and can be moved either along the vertical line (press CTRL key and left mouse button) or along the signal trace (left mouse button). The blue baseline points are fixed and cannot be moved. To highlight a baseline point, click it.



TIP

If you want to change several baseline points, deactivate the automatic analysis by clicking the *Pause Analysis* button in the toolbar. This way, the software will not recalculate the data analysis with every change. Once you have changed all baseline points, click the *Pause Analysis* button again to activate automatic analysis.

3 Adjust the baseline points as appropriate.



TIP

To move a peak baseline point along the vertical line, press the CTRL key and the left mouse button. To move a peak baseline point along the signal, press the left mouse button only.

4 Click the *Automatic Analysis* button to enable the integration again.

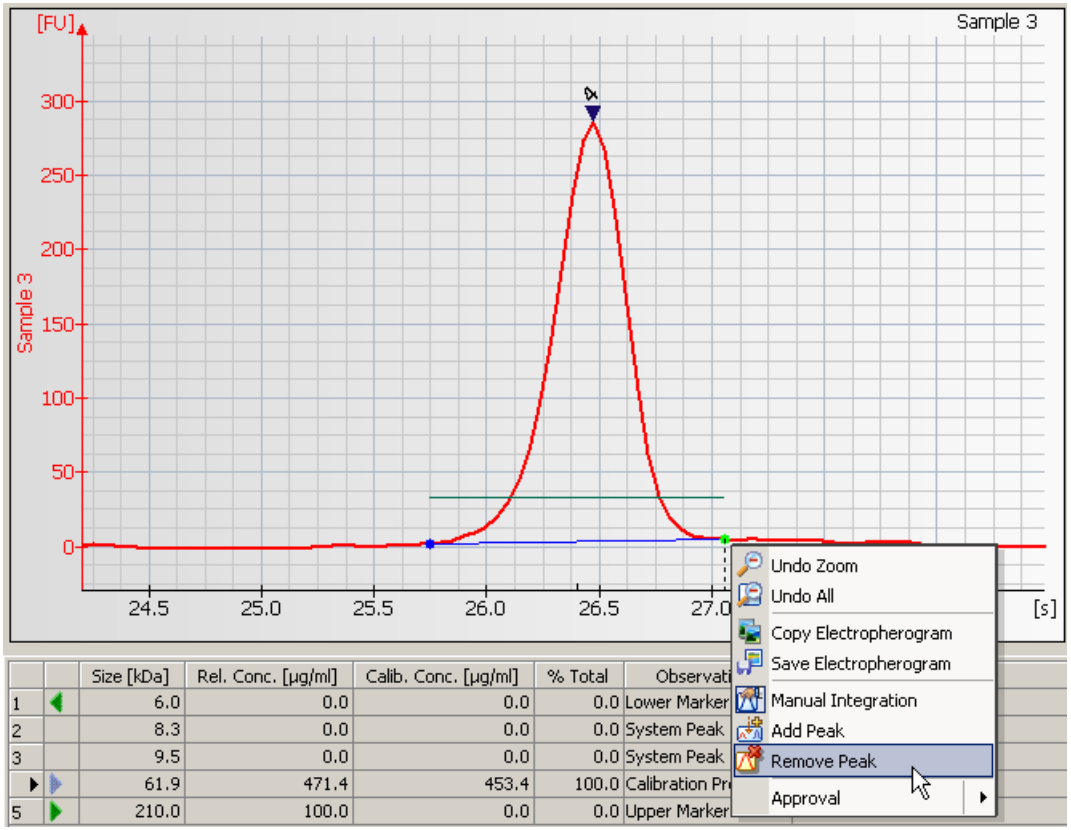
The integration results in the result and peak tables will change according to the changes done.

Example: Removing peaks

To remove peaks:

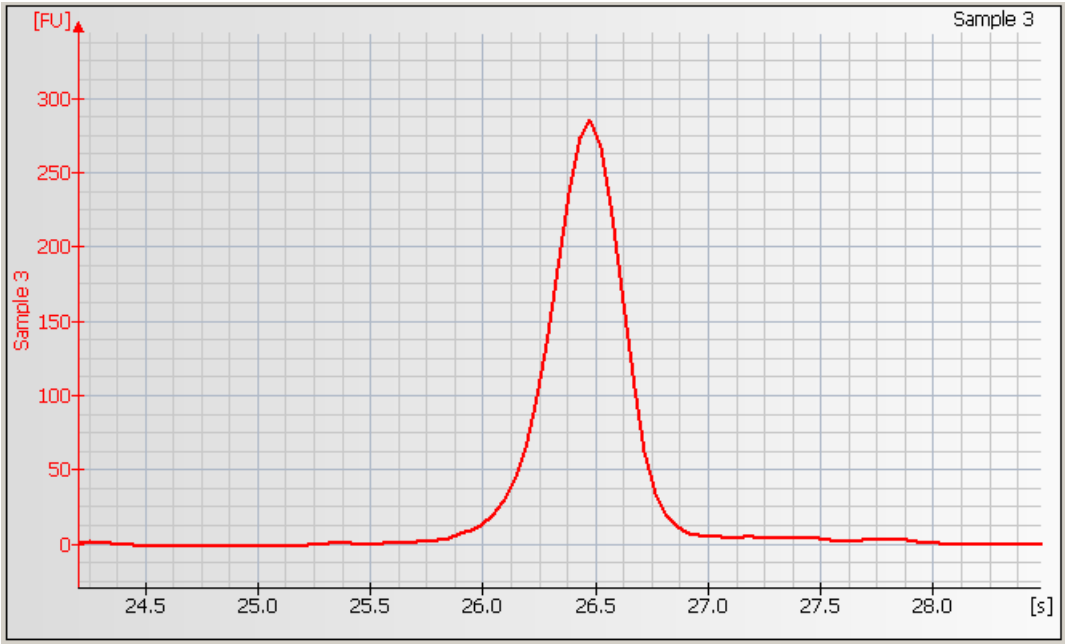
1 Highlight the *Electropherogram* tab in the *Data* context and zoom into the electropherogram to enlarge the peak of interest.

2 Select *Electropherogram* > *Manual Integration* to switch off the automatic integration. As an alternative you might click the *Manual Integration* button in the toolbar. The baseline points become visible as blue or green dots.



3 Right-click a baseline-point and select *Remove Peak* from the context menu.

The two baseline points and the connecting line will disappear and the integration results shown in the result and peak tables will be updated:

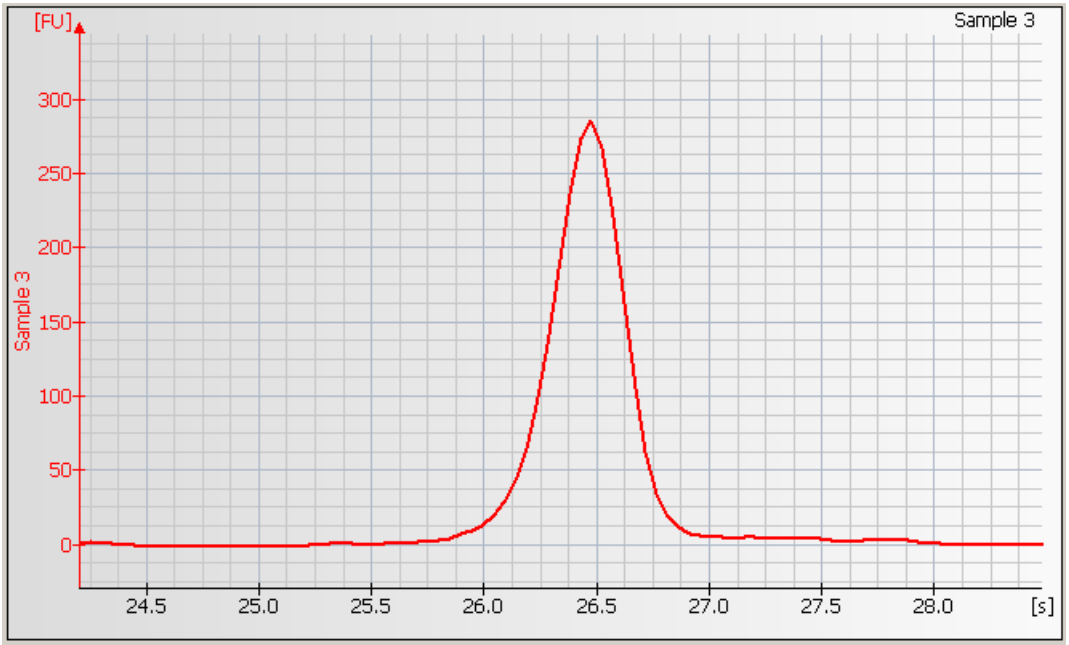


		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
		210.0	100.0	0.0	0.0	Upper Marker

Example: Inserting peak baselines

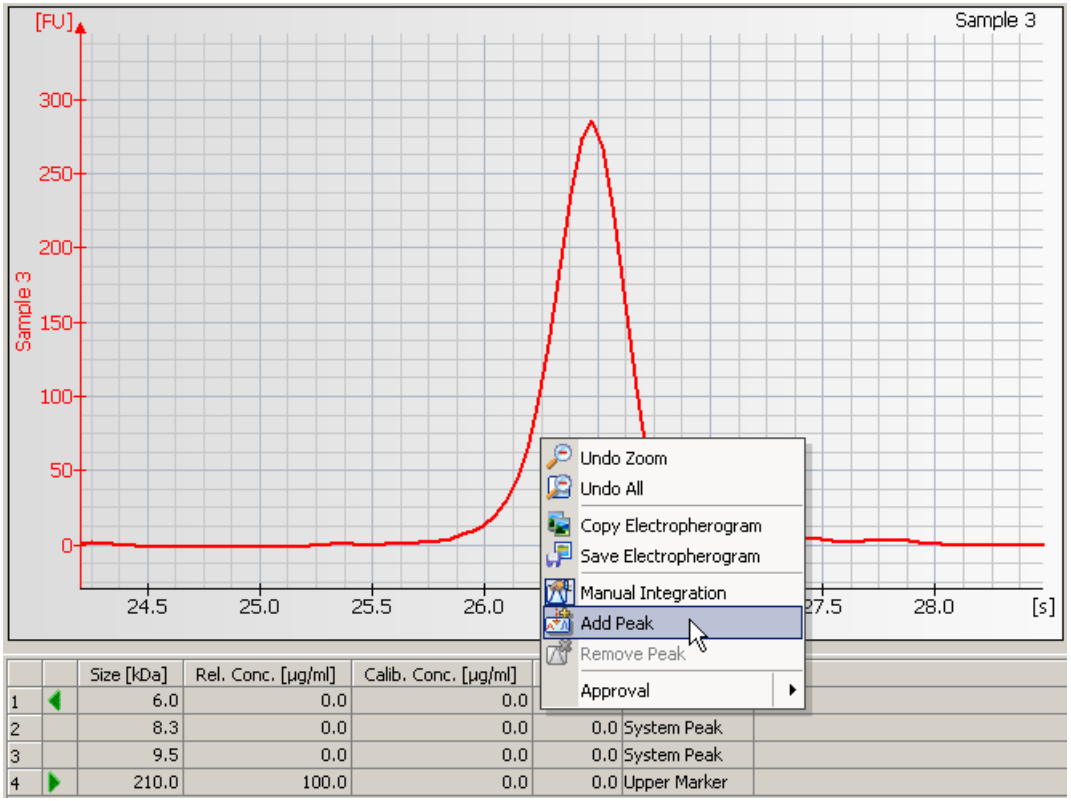
To insert peaks manually:

1 Highlight the *Electropherogram* tab in the *Data* context and zoom into the electropherogram to enlarge the peak of interest.



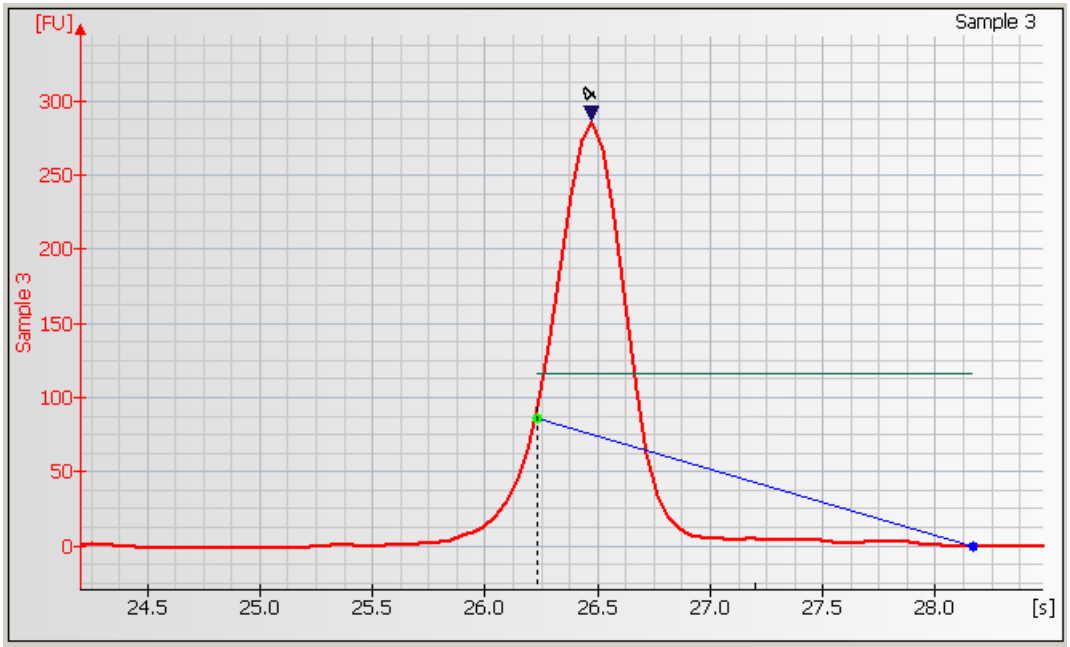
		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1	◀	6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
▶	▶	210.0	100.0	0.0	0.0	Upper Marker

2 Right-click the electropherogram and select *Add Peak* from the context menu.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]		
1	◀	6.0	0.0	0.0		
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
4	▶	210.0	100.0	0.0	0.0	Upper Marker

3 Two baseline points and the connecting line will appear and the integration results shown in the result and peak tables will be updated.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
		61.9	231.3	224.9	100.0	Calibration Protein
5		210.0	100.0	0.0	0.0	Upper Marker

TIP

If you want to change several baseline points, deactivate the automatic analysis by clicking the *Pause Analysis* button in the toolbar. This way, the software will not recalculate the data analysis with every change. Once you have changed all baseline points, click the *Pause Analysis* button again to activate automatic analysis.

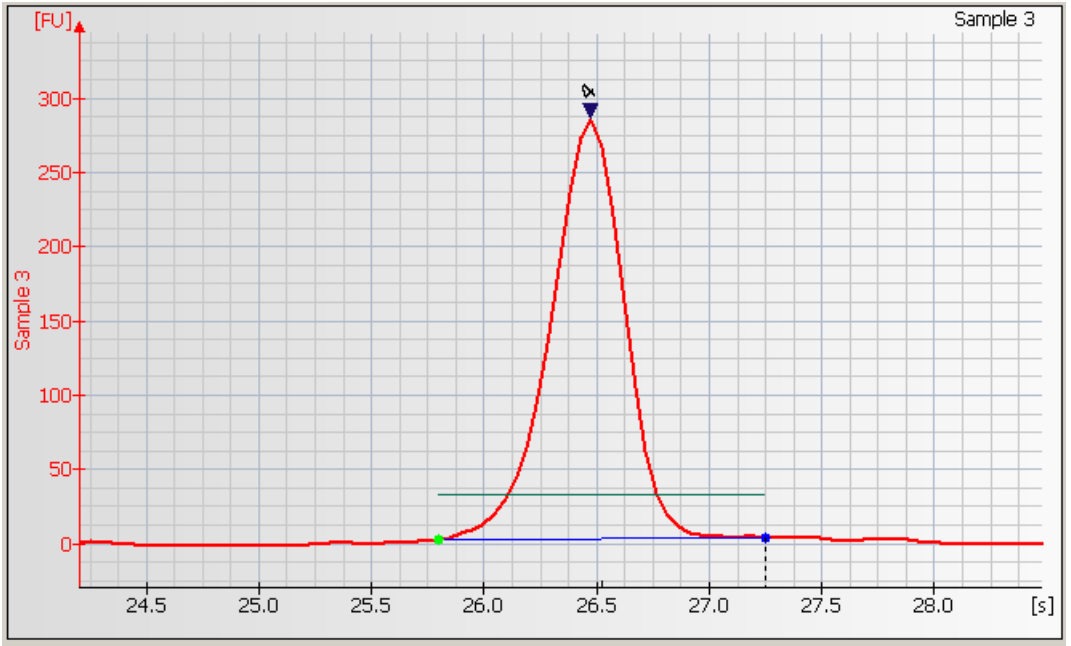
4 Adjust the baseline points as appropriate.

TIP

To move a peak baseline point along the vertical line, press the CTRL key and the left mouse button. To move a peak baseline point along the signal, press the left mouse button only.

5 Click the *Automatic Analysis* button to enable the integration again.

The integration results in the result and peak tables will change according to the changes done.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
		61.9	472.1	454.1	100.0	Calibration Protein
5		210.0	100.0	0.0	0.0	Upper Marker

Reanalyzing a Chip Data File

NOTE

Occasionally you may wish to open and view or reanalyze a chip data file that was run and saved previously. The raw data values are saved in the data file, along with the analysis settings that were chosen for the run, so that the data can be reanalyzed with different settings.

To do this:

1 Click *File* > *Open* to open a chip data file (.xad).

2. Choose the filename from the list of data files.

3. Click *OK*.

The items that can be changed for reanalysis are:

All Electrophoresis Assays:

- Global peak find settings
- Individual sample peak find settings
- Gel color
- Sample names and comments

RNA Assays Only:

- Fragment names and colors associated with labels
- Fragment start/end times, additional fragments (or delete fragments found)

- Reassign lower marker

DNA and Protein Assays Only:

- Exclude peaks from analysis
- Reassign upper/lower markers
- Alignment or no alignment with marker peaks
- Manual integration

Protein Assays Only:

- Absolute quantitation

TIP

When applying modified data analysis setpoints, the software will (by default) immediately recalculate the raw data, which takes some time. Select *Don't Analyze* from the *Gel Menu* or *Electropherogram Menu* to temporarily switch off automatic data analysis while you modify setpoints.

If you save the data file after making changes, it will keep a record of the changes such as gel color, sample names, and peak find settings that were in effect at the time the file is resaved. If you do not want to change the original file, choose *File* > *Save As...* and give the file a new name or save it to a different location.

Comparing Samples from Different Electrophoretic Chip Runs

The *2100 expert* software allows you to compare the measurement results of samples from different electrophoretic chip runs. Samples to be compared must be from chip runs of the same assay type.

In the *Comparison* context, you can create comparison files, include samples from different chip runs, and compare the samples by overlaying electropherograms, for example.

To compare samples from different electrophoretic chip runs:

1 Switch to the *Comparison* context.

2 From the *File* menu select *Open*, and open all chip data files (.xad) that contain the samples you want to compare.

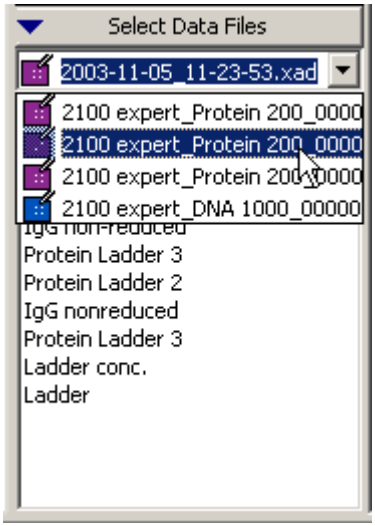
The .xad files appear in the *Select Data Files* list of the *Tree View Panel*.

NOTE

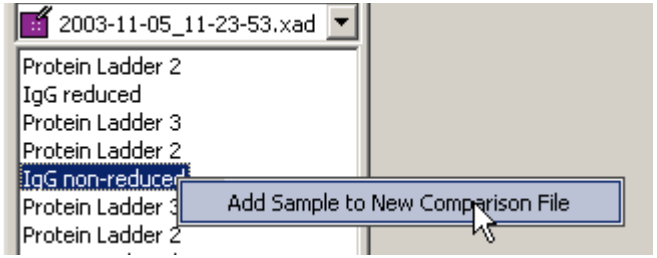
The *Select Data Files* list also contains all electrophoretic .xad files that are currently open in the *Data* context.

---

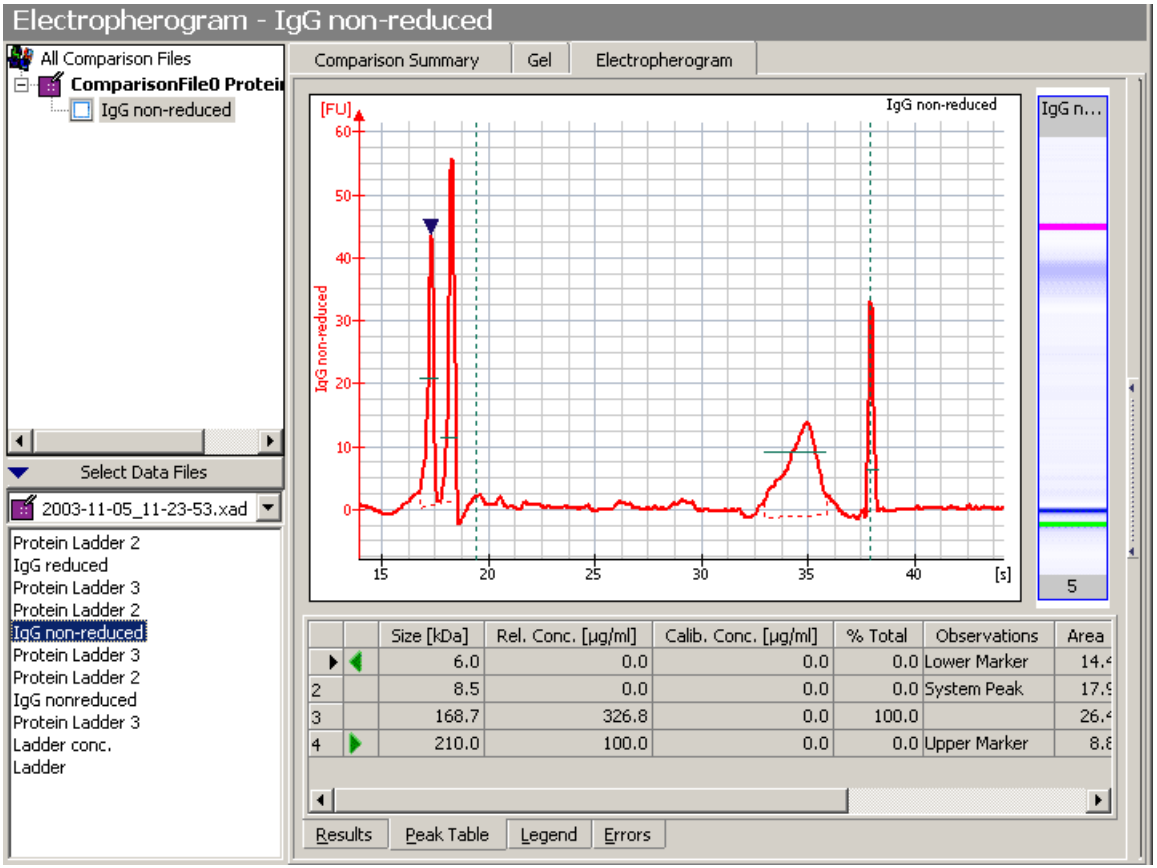
3 Select a .xad file from the *Select Data Files* list to display a list of its samples.



4 Right-click a sample name and select *Add Sample to New Comparison File*.

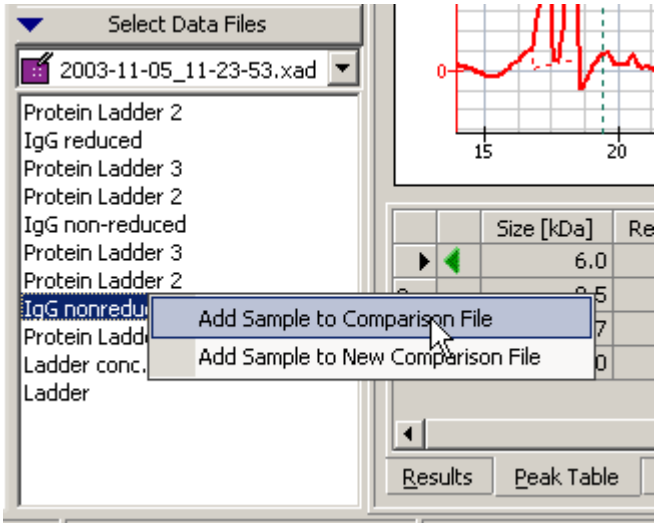


A new comparison file appears in the upper part of the tree view containing the sample. The sample is selected and its electropherogram is shown.



Note that the *Electropherogram Tab (Single/Grid View)* has the same functionality as in the *Data* context.

5 You can now add further samples from any of the open .xad files to the comparison file.

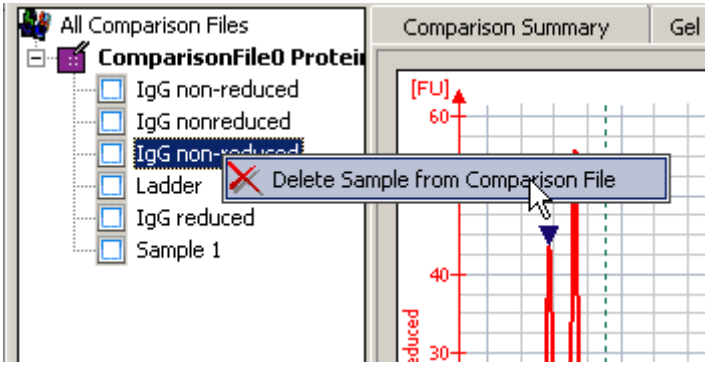


TIP

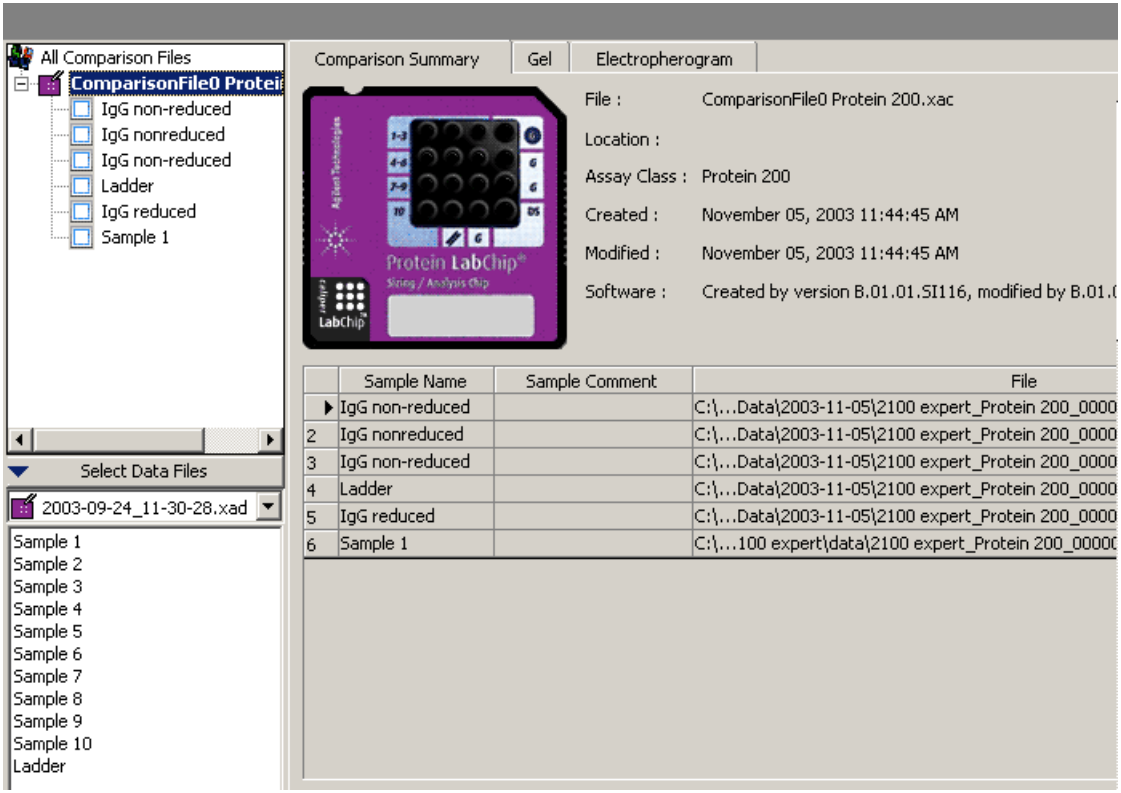
Double-clicking a sample name in the *lower* part of the tree view or dragging a sample name into the tree view adds the sample to the comparison file that is currently selected in the *upper* part of the tree view. Or, if no comparison file is selected, creates a new comparison file and adds the sample to it.

You are notified if you try to add a sample of a .xad file that has the wrong assay type.

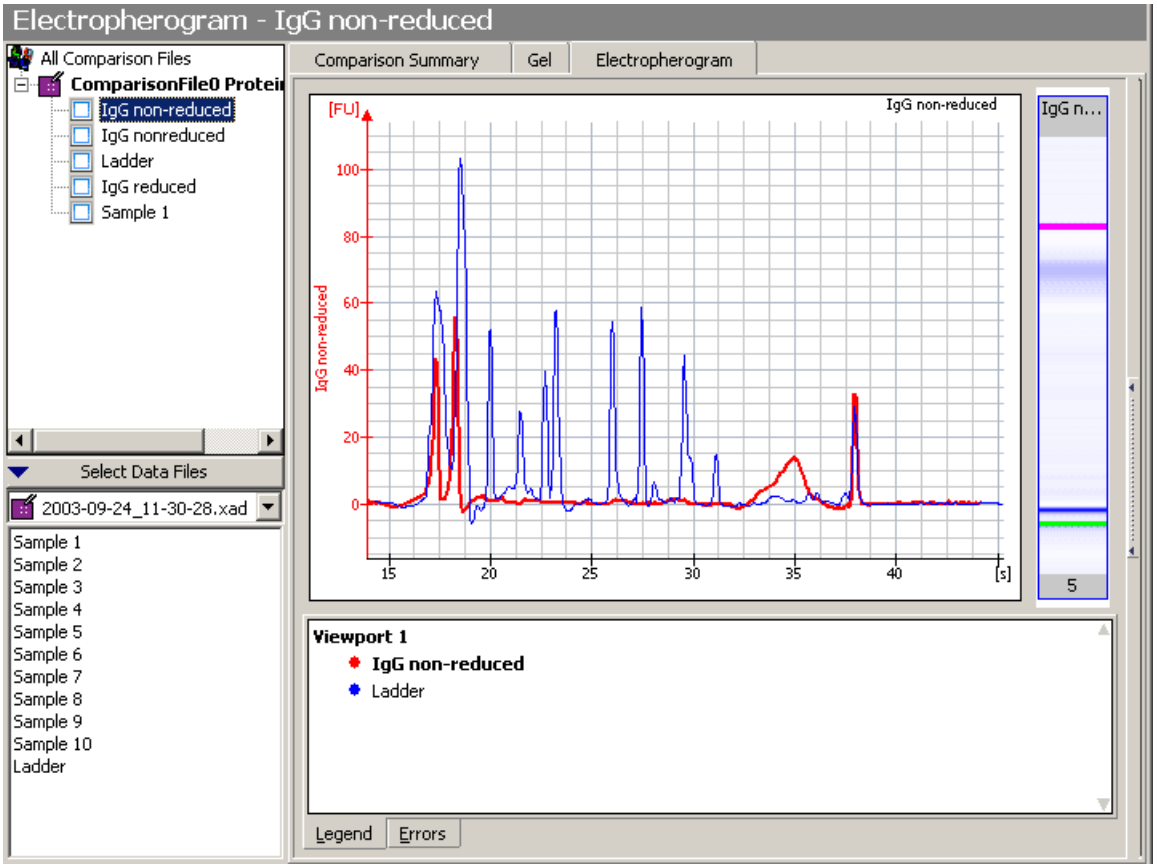
6 You can also remove samples from a comparison file. Right-click the sample name and select *Delete Sample from Comparison File*.



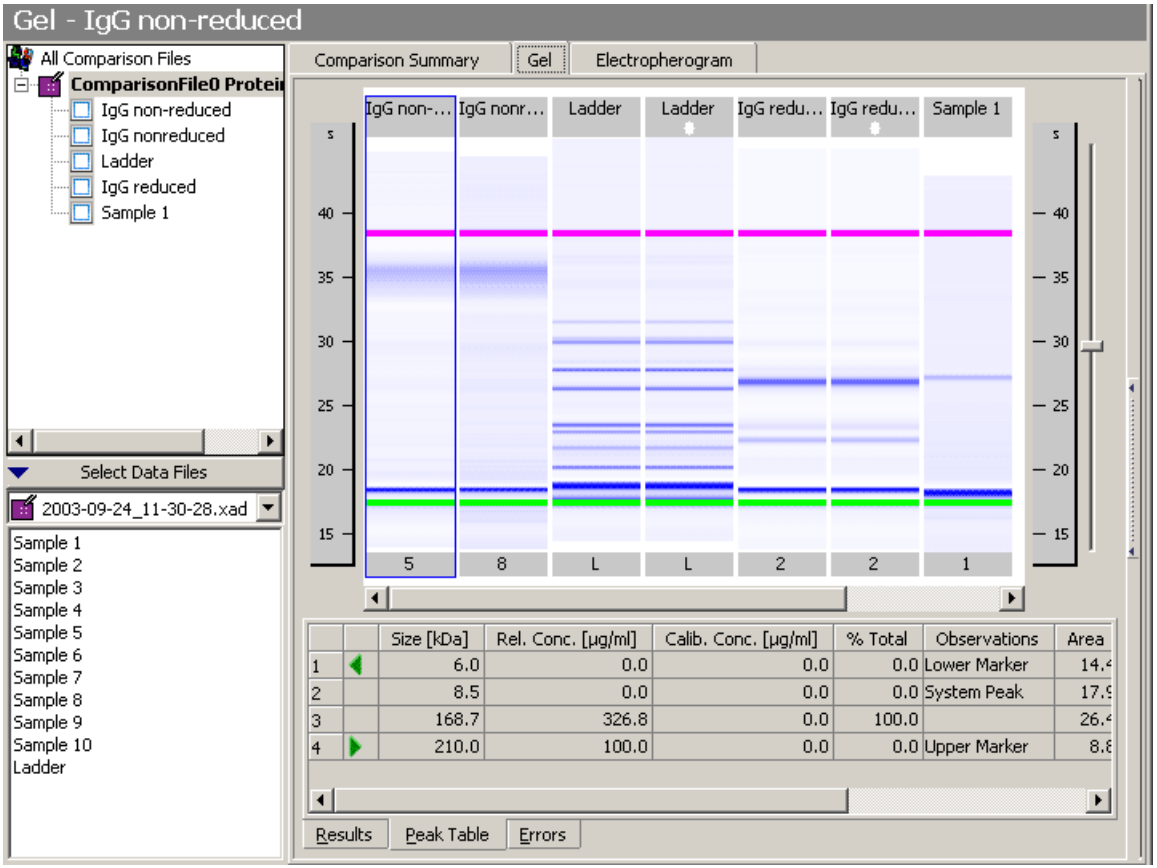
7 When you have added all your samples, you can select the *Comparison Summary Tab* which displays information on the comparison file, and lets you enter a comment regarding the comparison.



8 To compare the electropherograms of samples, go to the *Electropherogram* tab, click *Overlaid Samples* in the toolbar, and select the samples to be compared.



9 Select the *Gel* tab to display a comparison of the gel-like images of the samples.



Note that the *Gel Tab* has the same functionality as in the *Data* context.

10 From the *File* menu, select *Save* to save the comparison file (.xac) under the default name, or select *Save As* to save it under a new name.

The default name is derived from the assay class: "ComparisonFileX [Assay Class].xac" where "X" is an autoincremented number. Example: "ComparisonFile0 Protein 200.xac"

NOTE

You can re-open comparison files to review the comparison results, and to add/remove samples.

Result Flagging

Result flagging can be used to assign a user-defined color code to a sample. This lets you easily identify samples with certain properties immediately after a chip run.

The color assignment is carried out by applying a sequence of rules to the measurement results obtained for the sample. The rules are defined on chip level and are applied to all samples of the chip.

Two modes are available to define result flagging rules:

- Form Mode

  In this mode, you can easily compose an expression by selecting functions and operators from given lists. If necessary, additional attributes have to be provided.

  By selecting a logical operator (`AND/AND NOT/OR/OR NOT`), further terms can be combined to form a more complex expression. The last term of the expression ends with the operator `NONE`.

- Editor Mode

  This mode is more flexible and allows you to write arbitrary complex expressions by using functions, variables and operators.

TIP

You can export result flagging rules and import rules from other assay or chip data files. See "Exporting Result Flagging Rules" on page 285 and "Importing Result Flagging Rules" on page 276.

Regardless of how you create the result flagging rules, there are two options available for the order in which the rules are applied:

- In *Normal* mode, the rules are applied in the given order, and the first matching rule will determine the color of the sample. All rules are applied subsequently. The first rule which applies to the sample defines its color. So you should start with the most specific rule. If that one does not apply, a less specific one may apply. If none of the defined rules apply, the final default rule defines the color code.
- In *Target* mode, all rules are applied subsequently. If the next rule applies, the color code changes to the color code defined by the rule, otherwise the previous color code is kept. Therefore, the last matching rule defines the color code of the sample. This mode is called target mode because later rules refine the result color code. The first default color code is the most general and the last one the most specific.

You can define the flagging rules already in the assay, or—after the chip run is finished—modify these rules or define new rules in the chip data file, and apply the rules to the measurement results. Defined rules can also be saved, loaded and applied to other data files.

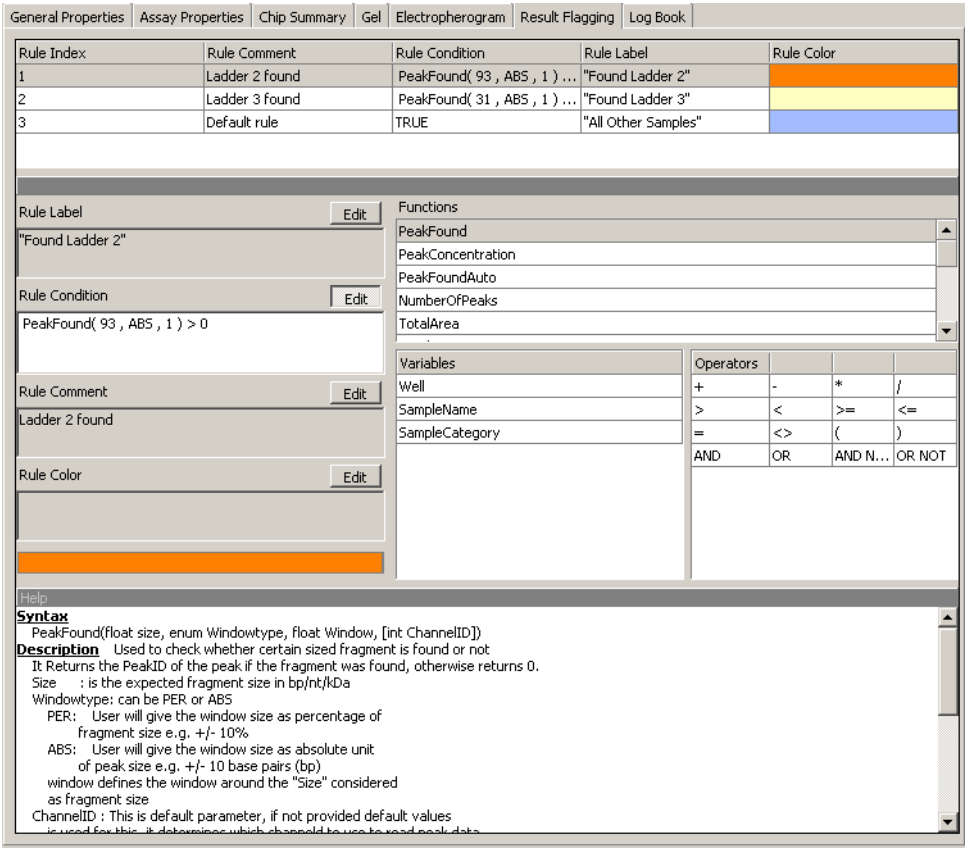
TIP

The examples shown in this chapter are taken from the demo assay “Demo Protein 200 Plus.xsy”, that comes with the *2100 expert* software. You can find this demo assay in the “..\assays\demo\electrophoresis” subdirectory of the *2100 expert* installation folder.

In the “..\data\samples\resultflagging” subdirectory of the *2100 expert* installation folder, you can find further examples for result flagging rules (.xml), which you can import in the “Protein 200 Plus” demo assay.

Defining Result Flagging Rules

The rules can be defined on the *Result Flagging* tab. This tab is available in the *Data* context if an electrophoretic chip data (.xad.) file is selected and in the *Assay* context if an electrophoretic assay (.xsy) file is selected.



A result flagging rule consists of the following:

- *Label Expression*

  An optional description for the rule used to label samples meeting this rule.

- *If Expression*

  An expression built from predefined functions, variables, and logical operators.

- *Comment*

  An optional comment for the rule.

- *Color Expression*

  A solid color or a color gradient built from two colors, used for flagging samples that meet the rule.

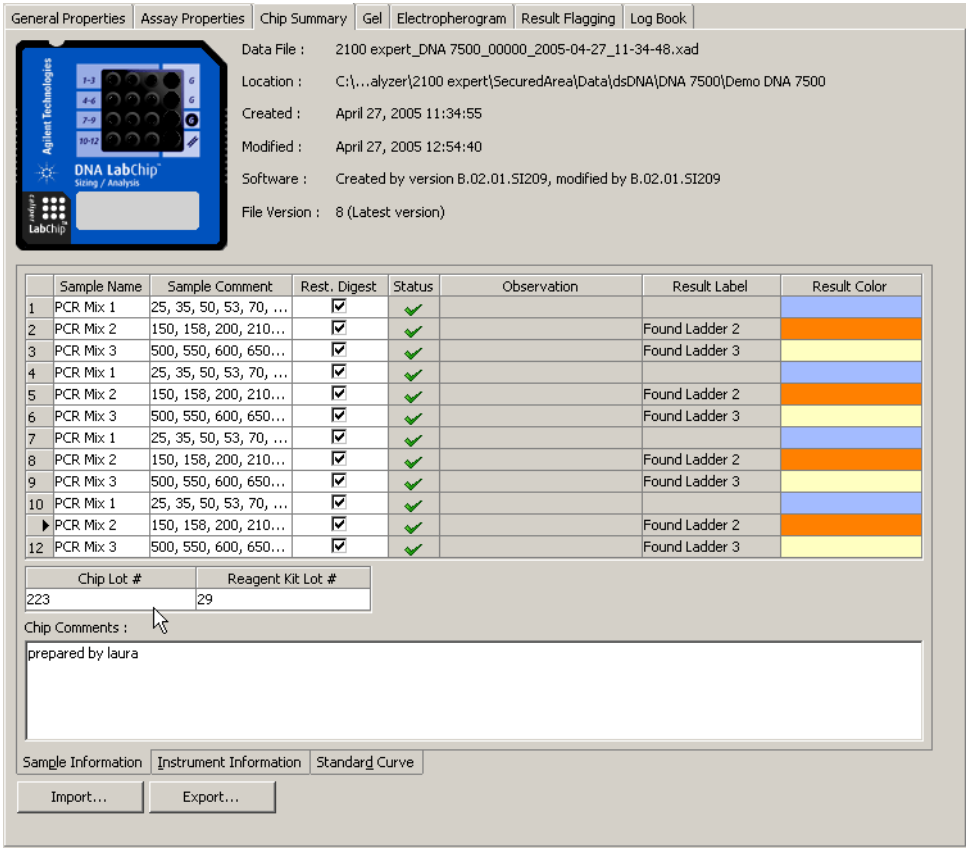
"How to Use the Form Mode" on page 159 shows how to proceed when defining rules.

You can reuse result flagging rule definitions, see "Exporting Result Flagging Rules" on page 285 and "Importing Result Flagging Rules" on page 276.

Color Indication

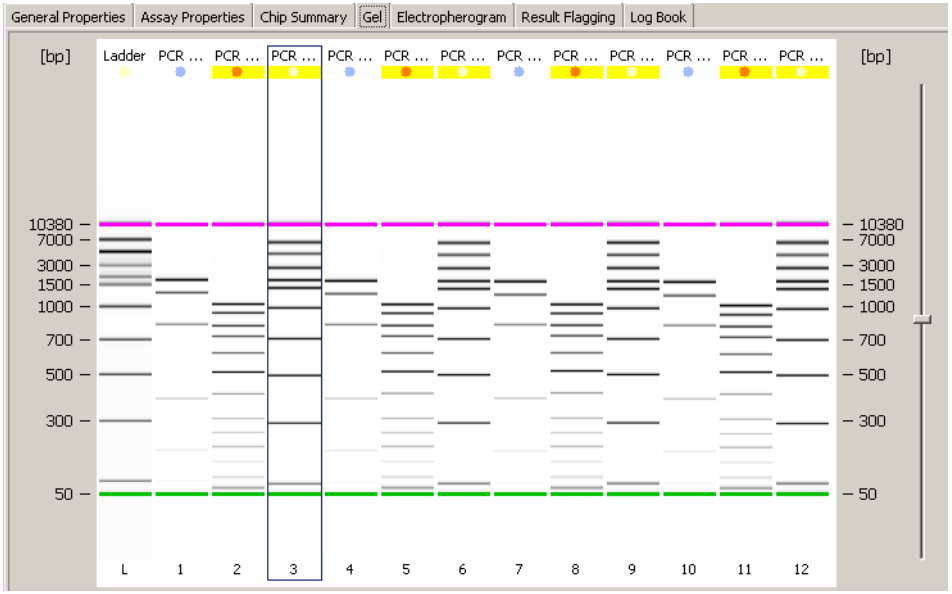
The results of the result flagging rules is displayed:

- On the *Chip Summary Tab:*


General Properties
Assay Properties
Chip Summary
Gel
Electropherogram
Result Flagging
Log Book
Agilent Technologies
DNA LabChip
Sizing / Analysis
Data File : 2100 expert_DNA 7500_00000_2005-04-27_11-34-48.xad
Location : C:\...alyzer\2100 expert\SecuredArea\Data\dsDNA\DNA 7500\Demo DNA 7500
Created : April 27, 2005 11:34:55
Modified : April 27, 2005 12:54:40
Software : Created by version B.02.01.SI209, modified by B.02.01.SI209
File Version : 8 (Latest version)
Sample Name | Sample Comment | Rest. Digest | Status | Observation | Result Label | Result Color
1 PCR Mix 1 25, 35, 50, 53, 70, ... Found Ladder 2
2 PCR Mix 2 150, 158, 200, 210... Found Ladder 3
3 PCR Mix 3 500, 550, 600, 650...
4 PCR Mix 1 25, 35, 50, 53, 70, ...
5 PCR Mix 2 150, 158, 200, 210... Found Ladder 2
6 PCR Mix 3 500, 550, 600, 650... Found Ladder 3
7 PCR Mix 1 25, 35, 50, 53, 70, ...
8 PCR Mix 2 150, 158, 200, 210... Found Ladder 2
9 PCR Mix 3 500, 550, 600, 650... Found Ladder 3
10 PCR Mix 1 25, 35, 50, 53, 70, ...
PCR Mix 2 150, 158, 200, 210... Found Ladder 2
12 PCR Mix 3 500, 550, 600, 650... Found Ladder 3
Chip Lot # Reagent Kit Lot #
223 29
Chip Comments :
prepared by laura
Sample Information
Instrument Information
Standard Curve
Import...
Export...

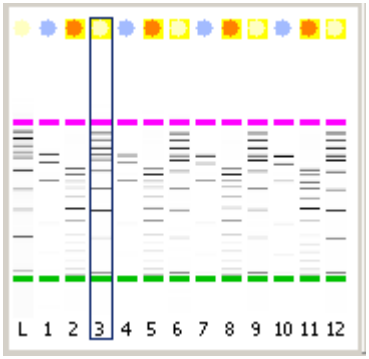
The colors in the *Result Flagging* column show which sample matches which rule.

- On the *Gel Tab:*

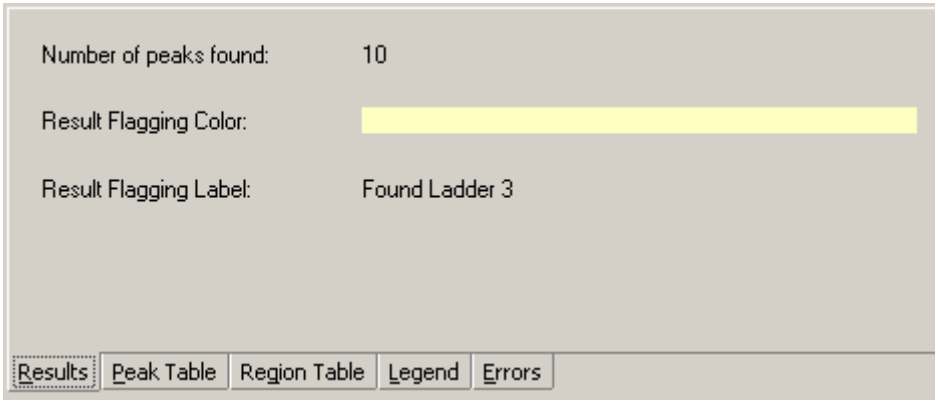


The spot on top of the lane is colored if the sample matches a result flagging rule.

- On the small gel image on the *Lower Panel:*



- On the *Results* tab:



Result Flagging Color: color of the result flagging rule that the current sample matches.

Result Flagging Label: label of the result flagging rule that the current sample matches.

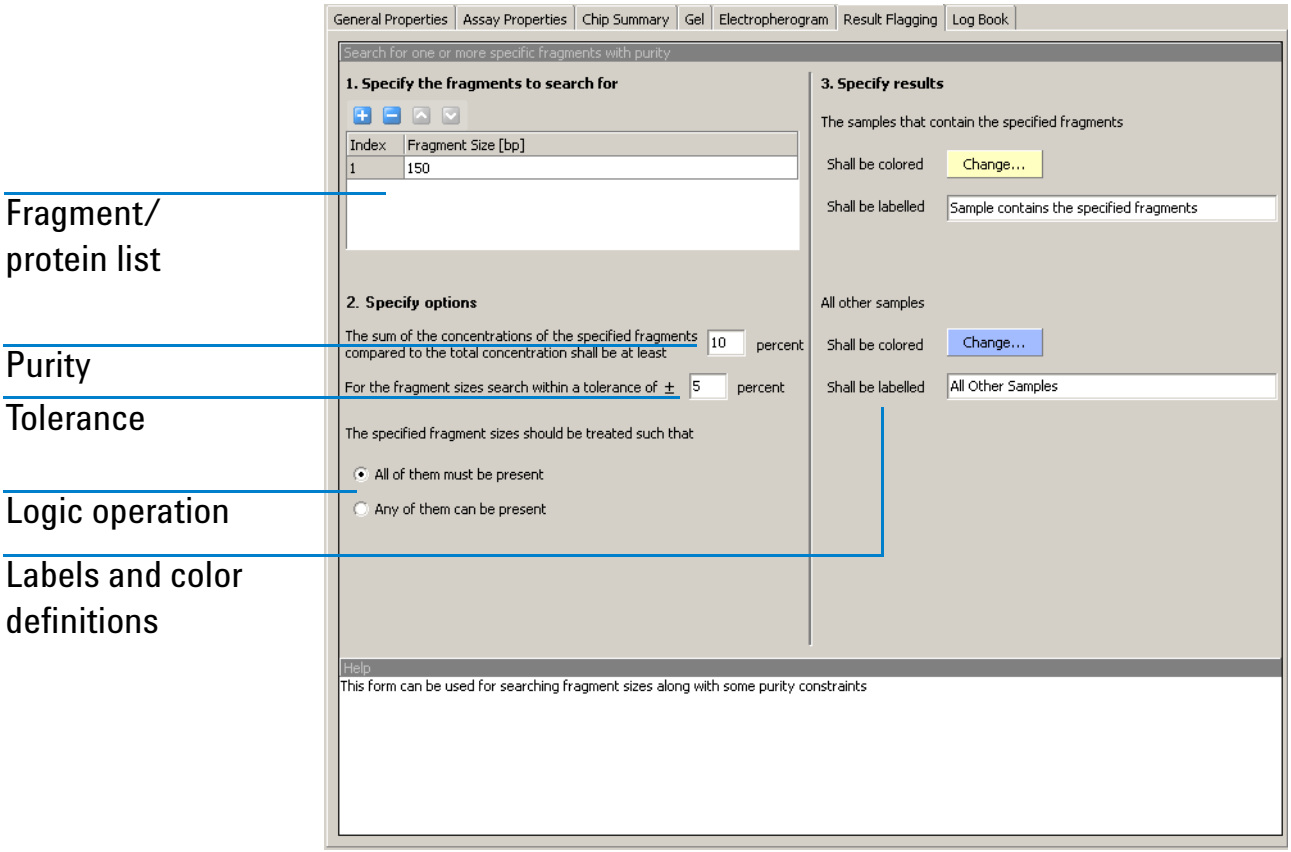
How to Use the Form Mode

The Form Mode provides some pre-defined rules (forms) that you can use to define the result flagging rules to color-code your samples. You can set up any number of rules for evaluation. As a typical example of how these forms are used, you can use the form mode to flag DNA samples that have a fragment purity of 10% for fragment sizes of 150 bp.

To do this, proceed as follows:

1. Open the job that contains the results to be analyzed in the *Data* context and switch to the *Result Flagging* tab.
2. Switch to the form mode by clicking the *Switch to Form Mode* icon .
3. Choose the *Search Fragment with Purity* form from the *Select Form* selection list.

The *Search Fragment with Purity* form is displayed.



4 Define the fragment size(s) to be searched for.

5 Define the required purity for the fragment size(s) and the tolerance.

6 If you defined several fragment sizes and want all of these to be present in the flagged samples, select the option *All of them must be present*. If you only require that one of the sizes is present, select the option *Any of them can be present*.

7 Select the color with which the samples that meet the criteria should be marked.

8 Optionally select the color with which samples that do not meet the criteria should be marked.

9 Apply this rule to the samples by clicking the *Apply Result Flagging* icon .

All samples are re-evaluated according to the result flagging rule and displayed with the respective colors. See "Color Indication" on page 157 for more information on the color codes.

Additional information is available in the *Help* panel at the bottom of the screen. This panel provides context-specific help, including examples.

How to Use the Editor Mode

The editor mode for result flagging is a powerful way of creating your own result flagging rules.

To define a result flagging rule for a selected job:

1 Open the job in question in the *Data* context and switch to the *Result Flagging* tab.

2 Switch to the editor mode by clicking the *Switch to Editor Mode* icon .

3 Edit a rule that was created within the form mode.

–OR–

Create a new rule by clicking the *Add* icon or *Duplicate Selected Rule* icon .

4 Click the *Edit* button next to the *Rule Label* field and enter the result label for this rule.

The result label can be any arbitrary text or be a logic expression. Expressions are built up of functions, variables, operators, and values. You can manually type in the expressions. But you can also double-click the items in the *Functions*, *Variables*, and *Operators* lists, to insert them in the respective fields.

For example, defining the result label as `"Number of peaks is" + NumberOfPeaks()` returns *Number of peaks is 10*, if the sample contains ten peaks.

Detailed descriptions of the available functions as well as the required syntax and examples are shown in the *Help* field at the bottom of the screen.

NOTE

If the entered syntax is not correct, the invalid part is displayed in red color.

---

5 Click the *Edit* button next to the *Rule Condition* field and enter the logic expression for this rule.

As an example for a logic expression for the rule condition, enter `NumberOfPeaks() > 9 AND PeakFoundAuto(150)`. With this rule, all samples can be found that have more than nine peaks while one of them is located at 150 bp +/- 10%.

6 Click the *Edit* button next to the *Rule Comment* field and enter a comment for this rule.

7 Click the *Edit* button next to the *Rule Color* field and select a color for the rule. If you check the *Gradient* check box, you can assign a color gradient to the rule. This allows to imply further information in the displayed results.

For example, the rule condition `NumberOfPeaks() > 0` marks all samples with peaks. If you want to indicate the actual number of peaks with the color code, you need to enter `NumberOfPeaks()` in the *Rule Color* field. Then you define light green for the *Minimum Value* 1 and dark green for 10 peaks as the *Maximum Value*. As a result, a darker green will be displayed for samples with more peaks.

8 If necessary, generate additional rules. Rearrange the order of the rules with the *Move Selected Rule Up* and *Move Selected Rule Down* icons.

9 To apply the rules to your measurement results, click the *Apply Result Flagging* icon .

If there still are syntax errors in the rule definitions, an error message appears.

All samples are re-evaluated according to the result flagging rules and displayed with the respective colors. See "Color Indication" on page 157 for more information on the color codes.

Example: Result Flagging

Sample 1 contains 100 µg/ml proteins. The electropherogram shows 2 peaks for 2 different proteins, which could be separated. One peak can be found at 32 kDa (LDH).

Sample 2 contains 60 µg/ml proteins and shows 3 peaks.

Sample 3 contains 80 µg/ml proteins and shows 5 peaks.

Now, the following rules are defined:

1. Is there a peak at 30 kDa +/- 7%?

   Rule 1: `PeakFound(30, PER, 7)`

2. Is the total concentration of proteins higher than 90 µg/ml?

   Rule 2: `TotalConcentration() > 90`

3. Were 5 to 10 peaks found?

   Rule 3: `NumberOfPeaks() >= 5 AND NumberOfPeaks <= 10`

   Alternative Rule 3: `NumberOfPeaks() BETWEEN (5,10)`

Rule Index	Rule Comment	Rule Condition	Rule Label	Rule Color
1		PeakFound(30, PER, 7)	"Any peak at 30 kDa +/- 7 %"	
2		TotalConcentration() > 90	"Total concentration > 90"	
3		NumberOfPeaks() >= 5 AND NumberOfPeaks() <= 10	"Number of peaks between 5 and 10"	
4	Default rule	TRUE	""	

Applying these rules in the given order (in *Normal* mode) leads to the following results:

	Sample Name	Sample Comment	Use For Calibration	Conc.[µg/ml]	Status	Observation	Result Label	Result Color
1	beta LG		☐	0	✓		Any peak at 30 kDa +/- 7 %	
2	beta LG		☐	0	✓			
3	beta LG		☐	0	✓		Number of peaks between 5 and 10	

For sample 1, rule 1 matches and defines the color. Rule 2 would also match, but is not checked, because the procedure stops at the first match.

For sample 2, none of the rules match, if there is no peak at 30 kDa +/- 7%. Therefore, this sample will get the default color.

For sample 3, only rule 3 matches and defines the color.

Running and Evaluating Flow Cytometric Assays

For running and evaluating flow cytometric assays, you need to know the following:

- "Principles of Flow Cytometric Measurements" on page 167
- "Overview of Flow Cytometric Assays" on page 176
- "Preparing and Running a Flow Cytometric Assay" on page 179
- "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 211

Principles of Flow Cytometric Measurements

Besides electrophoretic assays (DNA, RNA, and proteins), the Agilent 2100 bioanalyzer supports flow cytometric assays:

- First, cells are stained with two fluorescent dyes that correspond to biologically relevant parameters, as described in the application notes available for each assay. "Staining Cells" on page 168 explains the principle.
- Next, the stained cells are analyzed on the chip. They pass the detector in single file and are analyzed individually for their red and blue fluorescence intensities. The results are displayed as histograms or dot plots. Refer to "Cell Detection with the Agilent 2100 Bioanalyzer" on page 170 for a detailed explanation.

Staining Cells

With the 2100 expert software, you can differentiate several properties of a cell. The characteristics that are examined depend on the dye, which binds specifically to a cellular constituent or is metabolized by the cell to generate a fluorescent product. You usually use two dyes with different colors. Typically, one of the two dyes is used as reference dye to select the target cells (living/dead, cell line type, etc.). The second dye can be used to detect another characteristic of the cell.

Recommended dyes

The tables below list dyes that match to the detection optics specification (Excitation max: 470 & 630 nm; Emission max: 525 & 680 nm).

The following dyes are recommended for use as the *blue* stain:

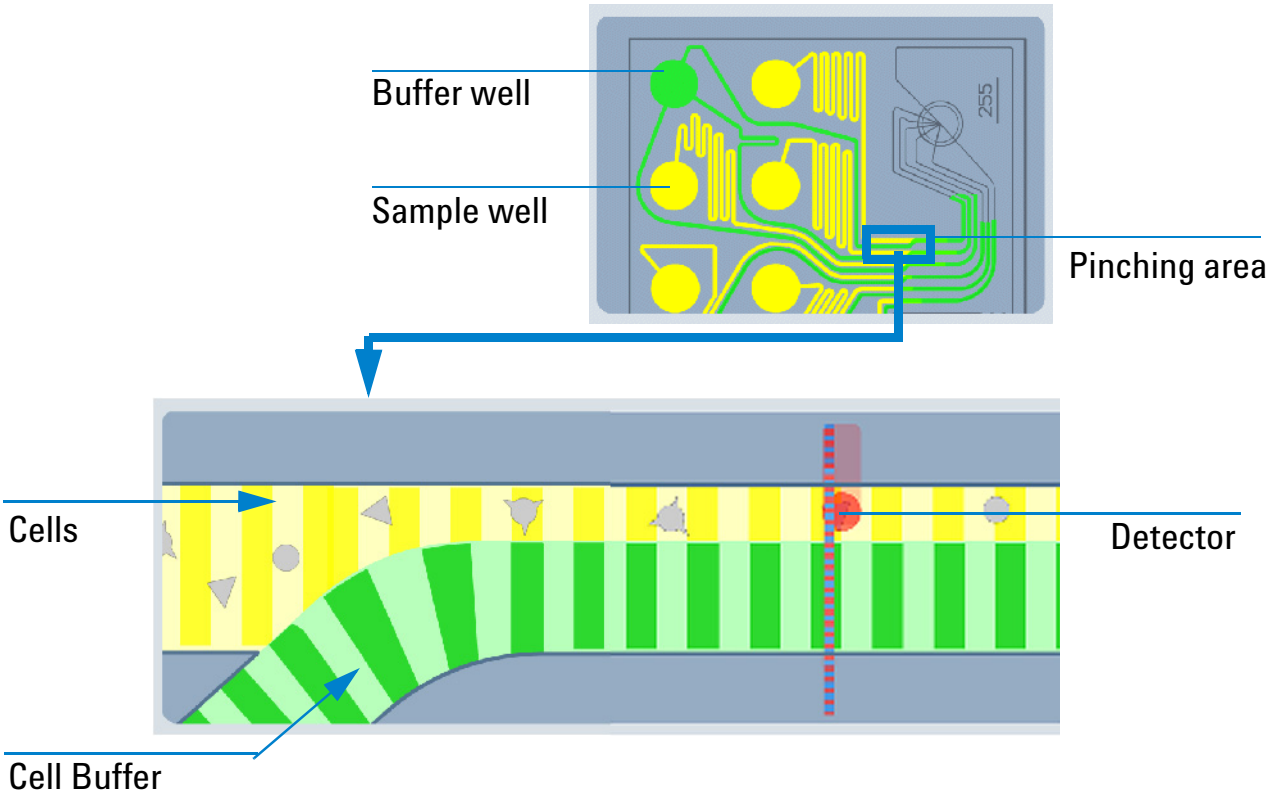
Dye (blue fluorescence)	Max. Excitation wavelength	Max. Emission wavelength
Calcein (living cell stain)	493 nm	514 nm
Cell Tracker green (cell tracing, viability stain)	492 nm	517 nm
GFP (green fluorescent protein)	490 nm	510 nm
SYTO16 (DNA dye)	485 nm	530 nm

The following dyes are recommended for use as the *red* stain:

Dye (red fluorescence)	Max. Excitation wavelength	Max. Emission wavelength
CBNF (Carboxynaphthofluorescein, living cell stain)	595 nm	675 nm
APC (Allophycocyanin, intra- and extra cellular antibody staining)	650 nm	660 nm
Cy5 (labeled Streptavidin and labeled anti-IgG, Apoptosis, intra- and extra-cellular antibody staining)	647 nm	665 nm
Alexa 647	650 nm	668 nm

Cell Detection with the Agilent 2100 Bioanalyzer

LabChip technology allows cell measurements by integrating cell flow, hydrodynamic focusing, and fluorescence detection into a microfluidic chip. A cell suspension can be confined or "pinched" to a portion of a microfluidic channel, causing cells to line up in single file for individual cell detection. The following images illustrate the pinching process.



Up to six cell samples can be analyzed on a chip. They are measured sequentially.

Measuring Events

The bioanalyzer counts cells stained with fluorescent dyes and measures their fluorescence intensities. Each cell or bead that passes the detector and emits fluorescence above a threshold value is counted as an event. For each event, the intensity of two different fluorescent signals (red and blue) is recorded. The intensity of the fluorescent signal depends on the amount of stain bound to the cell (and therefore a specific cell property) and the physical properties of the stain itself.

The Agilent 2100 bioanalyzer lets you determine the number of cells characterized by a specific pattern of fluorescence.

For example, to differentiate between dead and living cells, you can use a non-fluorescent dye that becomes fluorescent when metabolized by living cells. After staining with such a dye, living cells have a higher fluorescence value than dead cells. The second dye could bind to a specific surface marker on a subpopulation of the cells. This allows you to determine the number of living cells that contain your marker of interest.

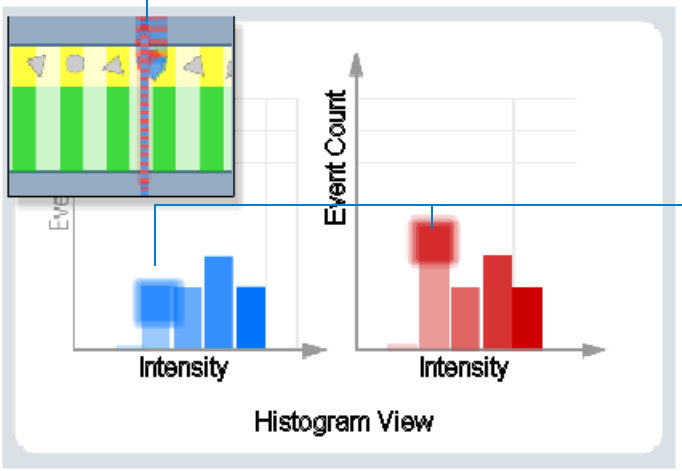
For evaluation, 2100 expert displays the results as histograms and as dot plots.

Generating Histograms

2100 expert counts the events, sorts them and displays them according to their fluorescence intensity in histograms. For each color measured, a histogram displays the number of events related to the fluorescence intensity. A large number of events with a high fluorescence value means that a large number of cells containing the fluorescence dye were detected.

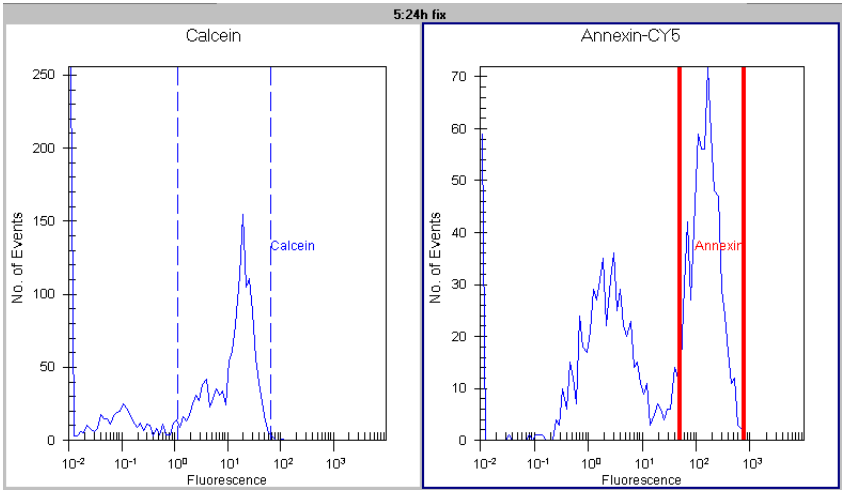
In the following illustration, cells which fluoresce in both colors are highlighted.

As a cell passes through the detector, its blue and red fluorescence values are measured and the count is increased for both channels at the appropriate intensity.



Illustrates a cell that fluoresces in blue and red. The height of the bars is related to the number of cells with this fluorescence value.

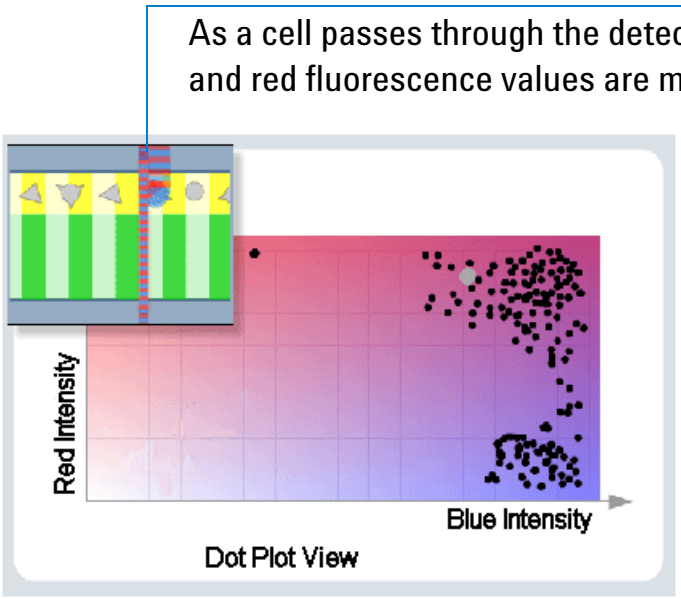
In the histograms, the bar chart is replaced by a point-to-point line as shown in the following image.



For detailed information, see "Using Histograms for Evaluation" on page 212.

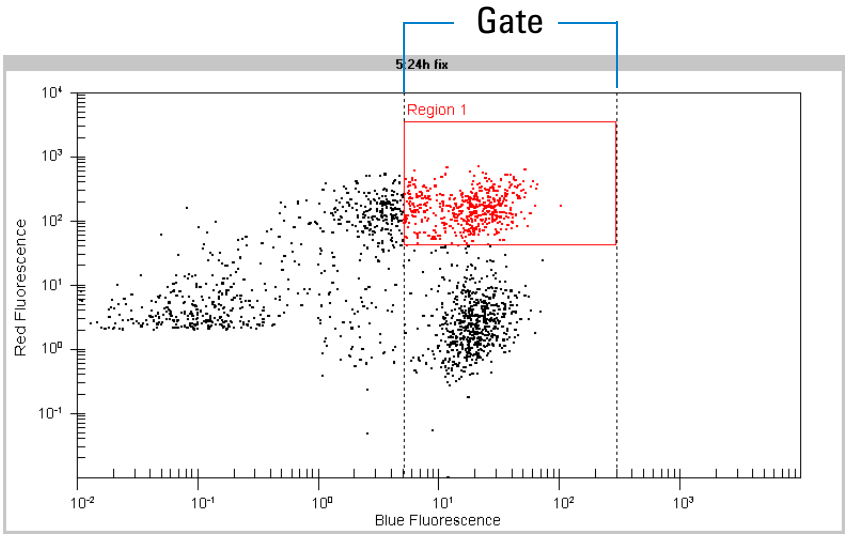
Generating Dot Plots

Single events can also be displayed related to both fluorescence values, generating a map of dot plots. In dot plot view, the events (cells with a minimum fluorescence intensity) are displayed in a coordinate system (logarithmic axis scaling). Each axis represents a fluorescence color. A high number of events (cells) with similar fluorescence values are displayed as a dense cluster of dots, as shown in the following image.


As a cell passes through the detector, its blue and red fluorescence values are measured.
Red Intensity
Blue Intensity
Dot Plot View

To determine the number of cells whose blue and red fluorescence lies within a defined range, you can insert regions. Additionally, a gate can be set for either red or blue fluorescence to obtain percentual values on a defined population.

In predefined assays, the borders of the rectangular region represent the markers defined in the corresponding blue and red histograms.



The lower left region of a dot plot usually shows no events, due to the defined peak detection threshold that the fluorescence values must exceed.

For detailed information, see “Using Dot Plots for Evaluation” on page 233.

Overview of Flow Cytometric Assays

The cell characteristic to be measured requires not only specific dyes. Several measurement parameters to control the measurement and the data acquisition parameters also have to be specified. These so-called “setpoints” are stored in assay files (.xsy) and are read by the 2100 expert software before it starts the measurement.

2100 expert supports the following assays based on flow cytometry:

Predefined assays

- *Apoptosis*
- *Apoptosis – fast protocol*

  For reduced background, this assay has an increased threshold and uses blue events only for peak detection.
- *Antibody Staining*
- *On-chip Antibody Staining*

  For reduced background, this assay has an increased threshold in the blue signal.
- *GFP*
- *On-chip GFP*

  This assay allows a rapid and accurate detection of green fluorescent protein expression.
- *Blue to red*

  This assay is for applications that apply a blue reference dye and analyze red fluorescent cells within a blue population.

- *Red to blue*

  This assay is for applications that apply a red reference dye and analyze blue fluorescent cells within a red population.

- *Checkout Beads*

  Red checkout beads are loaded into the wells 1, 3 and 5 and blue checkout beads into the wells 2, 4 and 6. Markers are set according to expected fluorescence levels of the red and blue beads. The *Checkout Beads* assay has the properties of a generic assay (see below).

- *siRNA Transfection Viability*

  Transfection Viability analysis as described in the Application Note: *siRNA transfection optimization with the Agilent 2100 bioanalyzer* (Agilent publication number: 5988-9782EN). This assay enables the automatic calculation of *transfection efficiency* (TE) in histogram view and *viability in transfected cells* (ViT) in dot plot view. Required gating directions and regions are provided as example, but can be adjusted. Final Transfection Viability (TV) can be calculated by multiplication TE and ViT values derived from histograms and dot plots.

The settings of predefined assays are optimized to measure the appropriate cell characteristics. For evaluation, it is only necessary to adjust the markers in histograms or regions in dot plots.

Predefined assays contain all necessary markers and regions for evaluation. The gating direction for histograms is given (for details on the gating direction, refer to "Gating" on page 214). The markers in all samples are connected (changing a marker changes the corresponding markers in all samples).

The regions of the dot plots are related to the markers of the histograms. Thus, the results of the dot plots are identical to the results of the histograms.

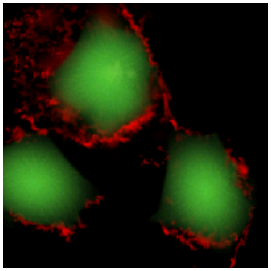
Generic assay

This assay has no specific settings and can be used to define individual assays. You can freely add markers or regions, and define the gating direction.

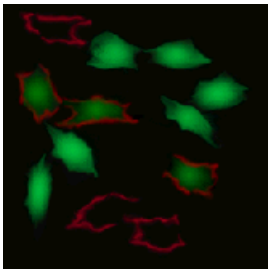
The generic assay is recommended for chips with different samples and stainings, where regions would need to be defined individually. Dot plot and histogram regions are not linked, making it possible to evaluate an individual sample with different settings.

Flow cytometry assay icons

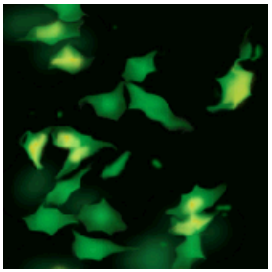
On the *Assay Properties* tab, the following icons are used to visualize the assay type:



Antibody Staining
Blue to Red



Apoptosis



GFP
Red to Blue

Generic
Checkout Beads
siRNA Transfection
Viability

Preparing and Running a Flow Cytometric Assay

A flow cytometric chip run requires the following steps:

1. Switch on the Agilent 2100 bioanalyzer and start the 2100 expert software.

   Details are given in "Starting 2100 Expert" on page 31.

2. Select a flow cytometric assay.

   See "Selecting a Flow Cytometric Assay for a Chip Run" on page 181.

3. Prepare chip and samples.

   Refer to "Preparing Samples and Chips for Flow Cytometric Assays" on page 185 and to the appropriate *Application Note* and *Reagent Kit Guide*.

4. Load the chip into the bioanalyzer.

   For details refer to "Loading the Cell Chip into the Bioanalyzer" on page 189.

5. Start the chip run.

   This is described in "Running a Flow Cytometric Assay" on page 196.

When the chip run has finished, you can:

- Have a first look at the results (see "Displaying the Measurement Results (Flow Cytometry)" on page 205).
- Document the chip run (see "Entering Chip, Sample, and Study Information" on page 202).

- Analyze and evaluate the results:
  - "Using Histograms for Evaluation" on page 212
  - "Using Dot Plots for Evaluation" on page 233
  - "Evaluating Antibody Staining, Apoptosis, and GFP Assays" on page 242

Selecting a Flow Cytometric Assay for a Chip Run

To select an assay:

1 Switch to the *Instrument* context.

2 In the *Tree View Panel*, select the bioanalyzer you want to use.

In the upper left of the *Instrument* tab, an icon shows the status of the bioanalyzer. You should see one of the following icons (lid open/closed), indicating that the bioanalyzer is detected by the system:

3 If you do not see one of these icons, check that the bioanalyzer is switched on and properly connected:

- Check the *COM port* setting.
- Make sure the bioanalyzer is physically connected to the PC (over the serial interface).
- Check the power connection.
- Check the power switch.

If you need additional help, please refer to the *Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide*.

4 Select an assay for the chip run.

On the *Instrument* tab, click the *Assay* button.

– OR –

Click the *Assays* menu.

Both will open the *Assays* menu, allowing you to select an assay from the submenus.

Note that you can also select *File* > *Open File to Run*. This opens a dialog box, allowing you to load either an assay (.xsy) or a chip data file (.xad).

The type of assay you have to select depends on the experiment and the staining protocol you use to prepare your cell samples. Details on these assays are described in the *Application Notes* available for each assay.

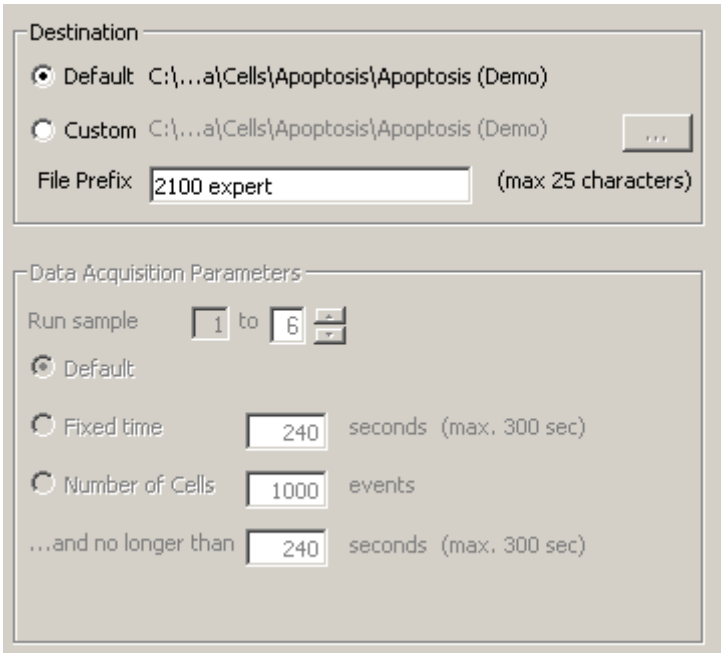
5 Select the desired assay, for example, *Apoptosis*.

The assay is loaded and its name appears on the *Information Bar*:

NOTE

After a chip run, the results can be evaluated using a different flow cytometric chip data or assay file. Refer to "Importing Data Analysis Setpoints" on page 273.

6 Select a *Destination* for the chip data file (.xad) that will be generated as the result of the chip run. You can also specify a custom *File Prefix* for this file.



7 If required, change the *Data Acquisition Parameters*:

a Enter the number of samples you want to be measured.

When preparing the chip (see "Preparing Samples and Chips for Flow Cytometric Assays" on page 185), keep in mind that you have to follow the sequence of the sample wells. For example, if you want to measure 3 samples, you have to fill the wells 1, 2, and 3 with your samples, and the remaining wells with cell buffer solution.

b Select the Data Acquisition Mode.

Select *Default*, if you want the measurement time to be set to the default value (240 s/sample). The maximum time is shown in brackets.

– OR –

Select *Fixed time* and enter the time in [s] that the measurement of each sample is to take.

– OR –

Select *Number of Cells* and enter the minimum number of *events* that should be measured. In the *and no longer than* field, enter the maximum time in [s] a measurement can take, regardless of whether or not the defined number of events is reached. The maximum time is shown in brackets.

NOTE

The overall run time for a chip is limited to 1440 s. The individual run time for one sample depends on the number of samples that are measured per chip. If only one sample is measured, you can set the run time up to 1440 s.

WARNING

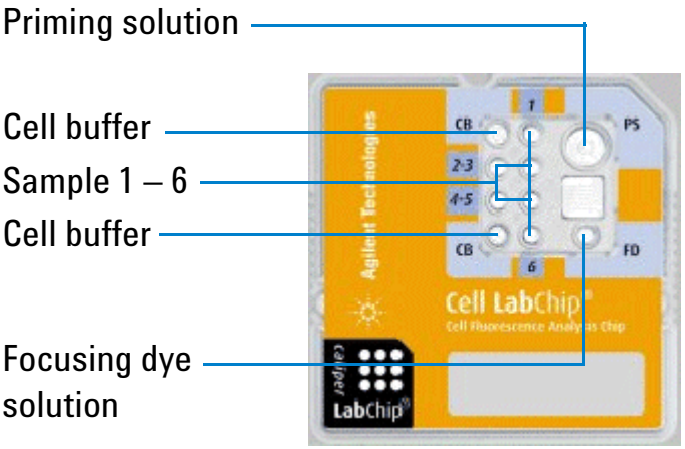
Several substances such as dyes can have toxic, carcinogenic, or mutagenic potential. Therefore, carefully follow the safety instructions from the dye safety data sheet and the *Reagent Kit Guides*. Also read the "Essential Measurement Practices (Flow Cytometric Assays)" on page 187.

Before you can fill a chip, you have to prepare the samples. To find out which protocols you should use to prepare the samples, refer to the various *Application Notes* available for each assay.

Sample and chip preparation is described in detail in the *Reagent Kit Guide* available for each LabChip kit.

Chip Reagents

Several reagents have to be added to the chip to prepare it for measurement. The following image shows which reagents have to be filled in which wells.



Make sure you follow these directions when preparing the sample:

- The priming solution has to be added first. It fills all channels (removes all air from the micro channels).
- The focusing dye is used to adjust the optic. The optics are focused horizontally and vertically before each chip is measured.
- If you do not use all six wells, always load the first sample in well 1, the second sample in well 2 etc. Unused wells have to be filled with cell buffer solution, otherwise they may run dry during the chip run. Because all channels are connected to the priming well, this may led to bubbles and to a clogging of the pressure adapter filter.
- The cell buffer is used to focus the cells before they pass the detection point. You have to fill both buffer wells with cell buffer.

Essential Measurement Practices (Flow Cytometric Assays)

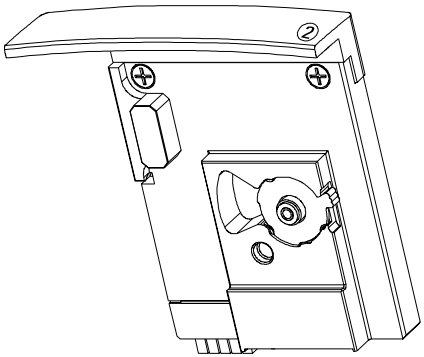
- Handle and store all reagents according to the instructions given in the *Reagent Kit Guides*.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Store all reagent and reagent mixes in the dark and refrigerated at 4 °C when not in use.
- Allow all reagents to equilibrate to room temperature for 30 minutes before use.
- Protect focusing dye from light. The dye decomposes when exposed to light.
- Use appropriate pipette tips. For each pipetting step use a fresh, new pipette tip.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results due to the formation of a bubble on the bottom of the well.

- For chip preparation, use inverse pipetting.
- When filling the pipette tip, push slightly over the first resistance. Empty the pipette tip only to the first resistance. This procedure avoids the introduction of bubbles and ensures pipetting the right volume.
- Never leave any wells empty, or the pressure adapter may become clogged. Pipette 10 µl of cell buffer or sample replicate in any empty sample well.

- Before bead preparation, vortex bead vials for 15 seconds.
- Prepared chips must be used within 5 minutes. If a chip is not run within 5 minutes, beads may settle or reagents may evaporate, leading to poor results.
- Never touch the instrument lens.
- Never touch the Agilent 2100 bioanalyzer during a chip run and never place it on a vibrating ground.

Loading the Cell Chip into the Bioanalyzer

The Agilent 2100 bioanalyzer uses different cartridges for electrophoretic and flow cytometric assays. For flow cytometric measurements, the *pressure cartridge* is required.



The pressure cartridge contains a tubing and filter assembly that connect to the vacuum pump. The seal has to match the priming well on the chip so that the required low pressure can be built up. The pressure cartridge can be identified by the engraved number “2” on the front.

NOTE

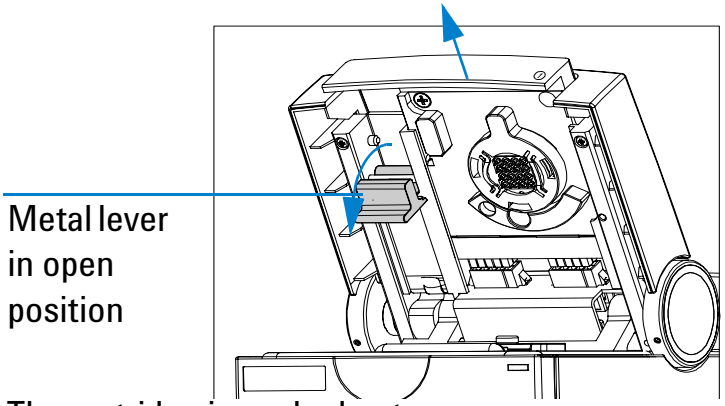
Any cartridges without engraved numbers are electrode cartridges.

If the bioanalyzer is set up for electrophoretic assays, but you want to run flow cytometric assays, proceed as follows:

1 Open the lid and pull down the metal locking lever in the open position as shown in the following figure.

CAUTION

Do not touch the electrodes while the cartridge is in the Agilent 2100 bioanalyzer. The electrodes and the high voltage power supplies can be damaged.



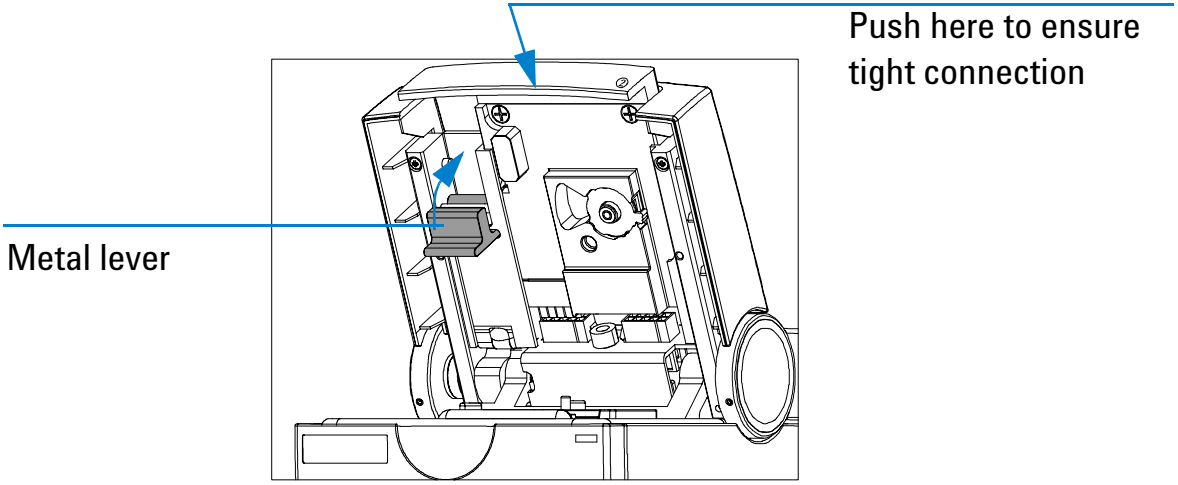
The cartridge is pushed out.

2 Gently pull the cartridge out of the lid.

CAUTION

Improper handling of the electrode cartridge will damage it.
Always store the electrode cartridge in the provided box. If the pins of the electrode cartridge are bent or misaligned, poor quality results or pre-terminated chip runs will result.

3 Slide the pressure cartridge in the lid as shown in the following figure.



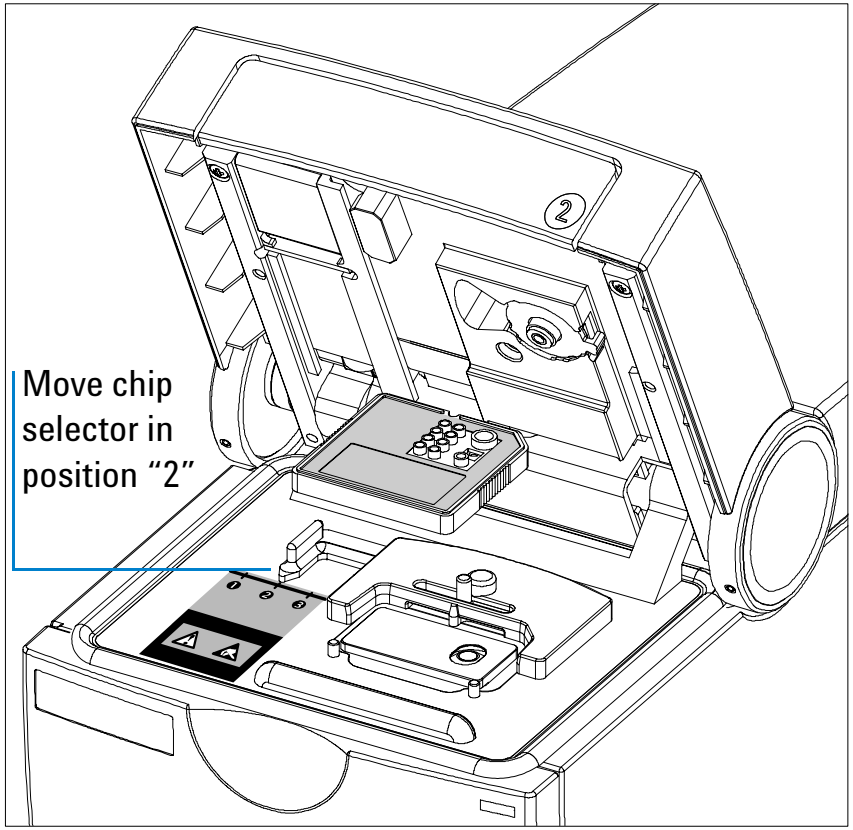
4 Push the metal front of the cartridge until it is securely in place.

5 Push the metal locking lever in the flat (closed) position.

To load the prepared chip into the Agilent 2100 bioanalyzer:

1 Open the lid and remove any chip.

2 Adjust the chip selector to position “2” as shown in the following figure.



To avoid using incompatible chips and cartridges, a chip selector is installed in the bioanalyzer. This ensures that the chip matches to the installed cartridge.

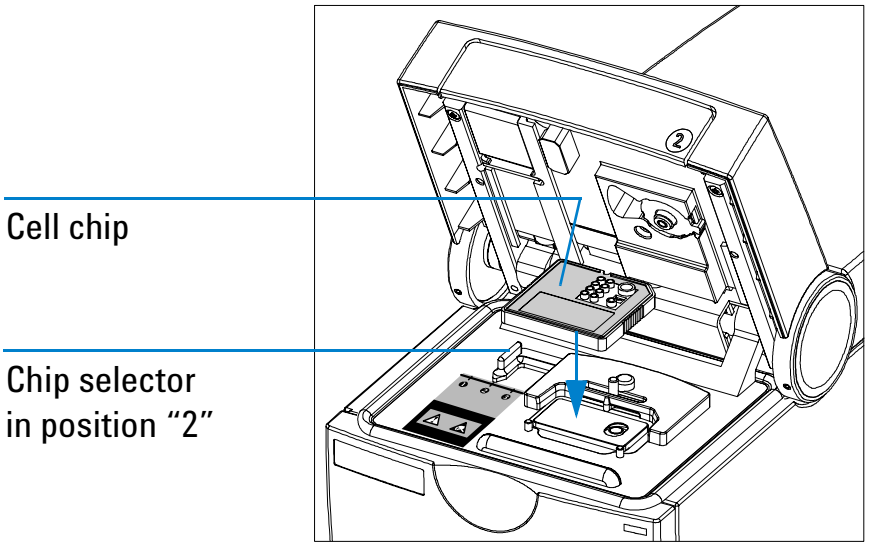
This will allow you to insert cell chips in the bioanalyzer.

CAUTION

Do not force the chip selector handle when a chip is inserted in the bioanalyzer.

3 Place the prepared chip into the receptacle.

The chip fits only one way. Do not use force.



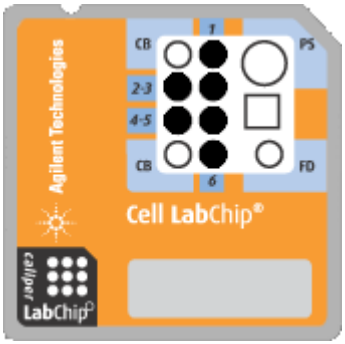
4 Carefully close the lid.

CAUTION

Do not force the lid closed. This can damage the pressure cartridge. If the lid does not close without force, check that chip is inserted correctly and that the chip selector is at the correct position for this chip type.

When the software recognizes an inserted chip, the chip is shown on the Instrument tab. If you have closed the lid, and the software has not recognized the chip, verify that the cartridge and chip are inserted properly and the chip selector is in the correct position. Close the lid.

The adapter with the gasket in the cartridge fits onto the priming well of the chip. A small gap between the lid and the instrument mainframe is normal and no cause for malfunction. The icon on the *Instrument* tab changes to a cell chip icon:



If the chip is not detected, open and close the lid again.

NOTE

If the *AutoRun* option is active, the chip run starts automatically once a chip has been inserted and the lid has been closed.

Running a Flow Cytometric Assay

Running a flow cytometric assay in 2100 expert just means pressing a button.

NOTE

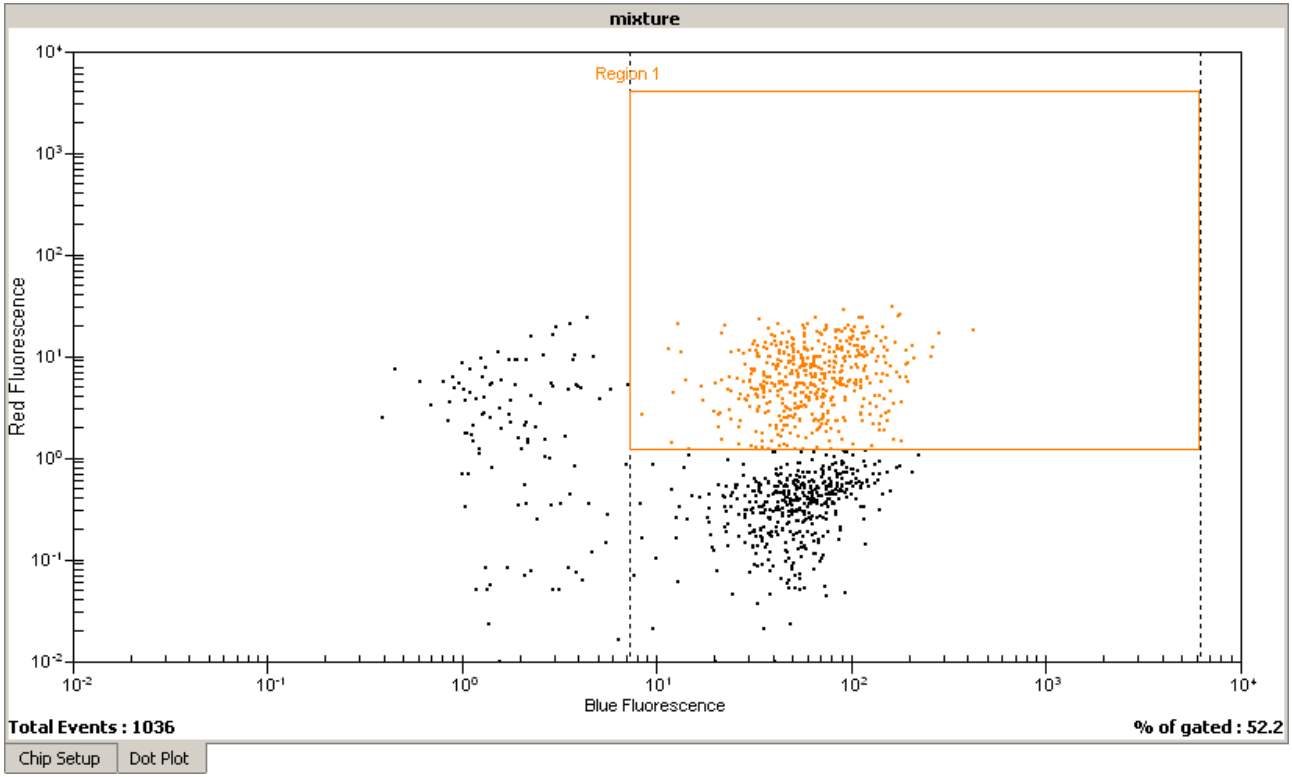
You can stop a chip run at any time, for example, if errors occurred, or if you are not satisfied with the quality of the measurement results, which you can observe during the chip run. See "Stopping a Chip Run" on page 200.

Starting the Chip Run

When you have loaded the chip, you can start the chip run:

1 On the *Instrument* tab, click the *Start* button.

The chip run starts. The *Dot Plot* sub-tab shows single events (cells) as they are detected, displayed as dots. In the coordinate system, the red and blue fluorescence intensity of each event can be read. The name of the currently measured sample is displayed above the graph.

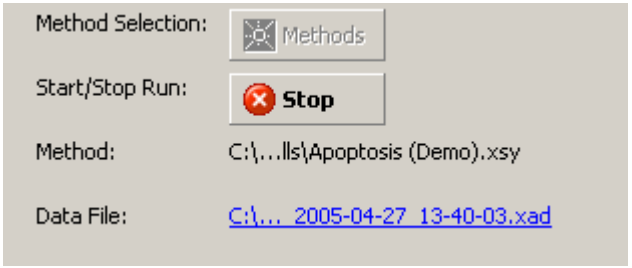


The number of the sample that is currently being measured is indicated on the information bar:

The status bar at the bottom of the screen shows the measurement progress for the chip run and the COM port number used for data acquisition.

During the chip run, you can do the following:

- View the chip data file in the *Data* context by clicking on the name of the *Data File*:



- Evaluate any chip data file in the *Data* context.
- Compare samples in the *Comparison* context.
- If necessary, abort the chip run by clicking on the *Stop* button (see also "Stopping a Chip Run" on page 200).

  All data that was collected up to the stop point will be saved.

After the chip run is completed, you can:

- Switch to the *Data* context, where you can view, analyze, and evaluate the results of your chip run (see "Displaying the Measurement Results (Flow Cytometry)" on page 205 and "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 211).
- Stay in the *Instrument* context, where you can start a new chip run, for example.

Stopping a Chip Run

You can stop a chip run at any time, for example,

- if the quality of the measurement results does not meet your expectations,
- if, for example, after three samples you already have the information you desired and you want to start another chip run.

NOTE

You *cannot* resume a stopped chip run.

NOTE

If you stop a chip run, automatic export (see "Exporting Chip Run Data Automatically" on page 280) and automatic print (see "How to Turn on and Configure Automatic Printing of Chip Run Reports" on page 291) does *not* take place.

To stop the assay:

1 Click the *Stop* button.

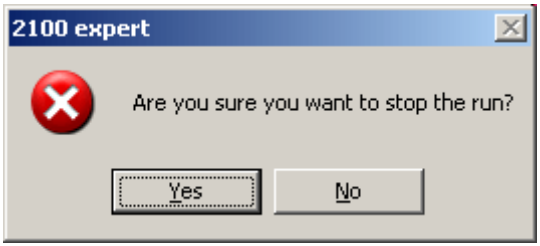
– OR –

Select *Stop* from the *Instrument* menu.

NOTE

Data acquisition of the current sample will be aborted.

The following message appears:



2 Click *Yes* to stop the chip run.

When the chip run is aborted, you can:

- Switch to the *Data* context, where you can view, analyze, and evaluate the results (if any) of your chip run (see "Displaying the Measurement Results (Flow Cytometry)" on page 205 and "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 211).
- Stay in the *Instrument* context, where you can start the next chip run, for example.

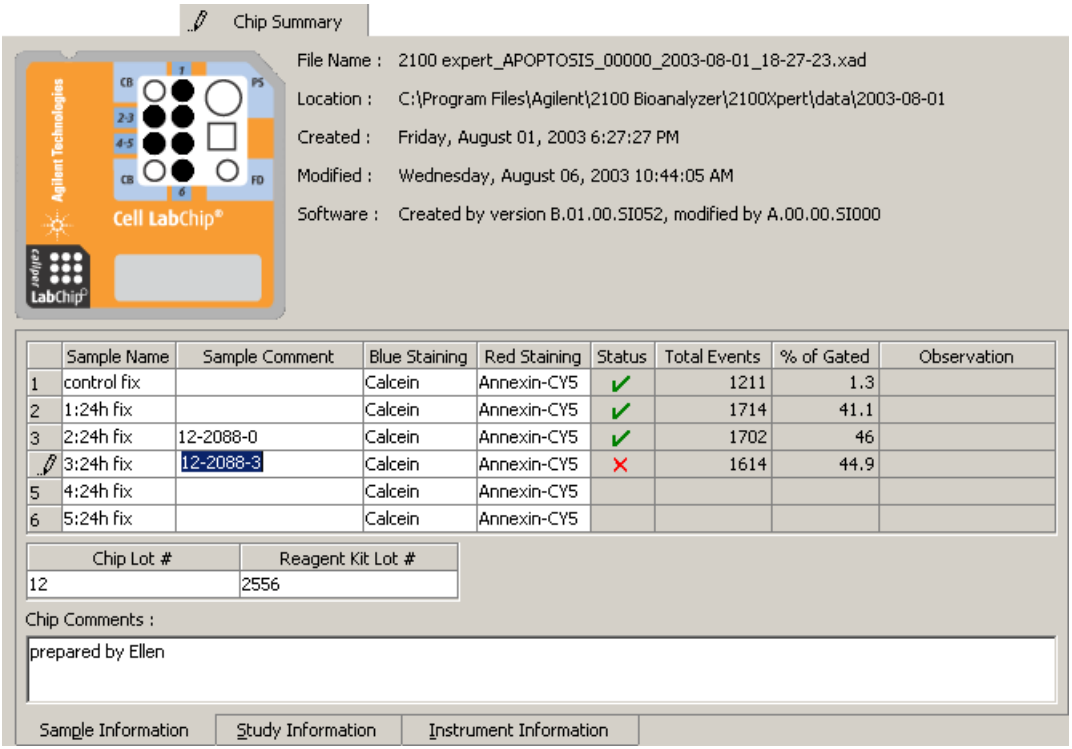
Entering Chip, Sample, and Study Information

During or after a chip run, you can document the run by entering information on chip, samples, and study.

1 In the *Data* context, select the *Chip Summary* tab.

2 On the *Sample Information* sub-tab, you can enter additional information for samples, such as names for blue and red stain.

On the *Study Information* sub-tab, you can enter information such as the name of the current study, the laboratory location, and the experimenter.


Chip Summary
File Name : 2100 expert_APOPTOSIS_00000_2003-08-01_18-27-23.xad
Location : C:\Program Files\Agilent\2100 Bioanalyzer\2100Xpert\data\2003-08-01
Created : Friday, August 01, 2003 6:27:27 PM
Modified : Wednesday, August 06, 2003 10:44:05 AM
Software : Created by version B.01.00.SI052, modified by A.00.00.SI000
Agilent Technologies
Cell LabChip®
LabChip®
Sample Name | Sample Comment | Blue Staining | Red Staining | Status | Total Events | % of Gated | Observation
1 control fix | Calcein | Annexin-CY5 | 1211 | 1.3
2 1:24h fix | Calcein | Annexin-CY5 | 1714 | 41.1
3 2:24h fix 12-2088-0 | Calcein | Annexin-CY5 | 1702 | 46
3:24h fix 12-2088-3 | Calcein | Annexin-CY5 | 1614 | 44.9
5 4:24h fix | Calcein | Annexin-CY5
6 5:24h fix | Calcein | Annexin-CY5
Chip Lot # | Reagent Kit Lot #
12 | 2556
Chip Comments :
prepared by Ellen
Sample Information | Study Information | Instrument Information

NOTE

You may find some input fields already filled in, because chip, sample, and study information are taken over from the base assay or chip data file.

3 From the *File* menu, select *Save*.

TIP

You can import chip, sample, and study information from .txt or .csv files. This is especially helpful and time-saving, if you already have documented a similar chip run in another chip data file. Refer to "Importing Chip, Sample, and Study Information" on page 275 for details.

Displaying the Measurement Results (Flow Cytometry)

You can view the measurement results of a flow cytometric chip run as histograms or dot plots.

- You can display the histograms/dot plots either one sample at a time, or all samples at the same time to get an overview of the chip run, for example, to see the progress of a reaction. See "How to Switch Between Single View and Grid View" on page 206.
- You can navigate through the samples. See "How to Navigate Through the Samples" on page 207.
- You can change the display of histograms and dot plots to make details better visible. See "How to Change the Display of Histograms and Dot Plots" on page 208.

How to Switch Between Single View and Grid View

To switch between single view and grid view:

1 From the *Histogram* or *Dot Plot* menu, select *Single View* or *Grid View*.

– OR –

Click the *Single View* or *Grid View* button on the histogram/dot plot toolbar.

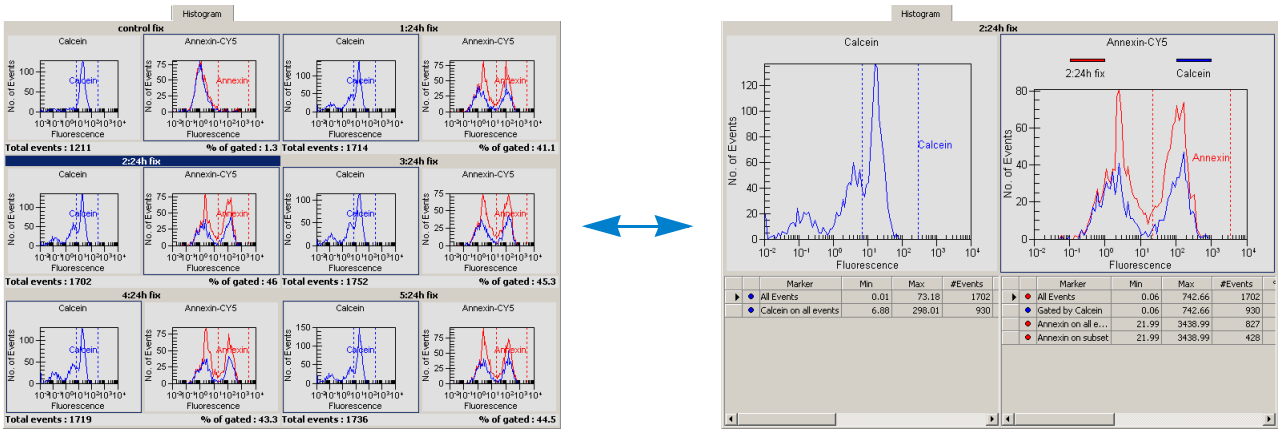
– OR –

Click the *All Samples* entry in the *Tree View Panel* to switch to the grid view, or any sample to switch to the single view.

– OR –

Double-click any histogram or dot plot in the grid view to switch to single view.

The following example shows switching between grid view and single view for histograms.



How to Navigate Through the Samples

At any time—even during a chip run—you can scroll though all samples—either in histogram or dot plot view.

To navigate through samples using the *Tree View Panel*:

1 If the tree view is not visible, select *View* > *Tree View*.

The Tree View Panel appears to the left of the tabs, and shows all chip data and assay files as nodes.

2 Click any sample name.

The histogram or dot plot of the sample is shown in single view.

To navigate through samples using the *Lower Panel*:

1 If the lower panel is not visible, select *View* > *Lower panel*.

The lower panel appears in the lower left corner, showing a chip icon.

2 Click any well on the chip icon.

To browse through samples:

1 From the *Histogram* or *Dot Plot* menu, select *Next Sample* or *Previous Sample*.

– OR –

Click the *Next Sample* or *Previous Sample* button in the histogram/dot plot toolbar.

To switch between histogram and dot plot view:

1 Click the *Histogram* or *Dot Plot* tab to display the results of the selected sample as a histogram or dot plot.

How to Change the Display of Histograms and Dot Plots

In *single view*, it is possible to change the display of histograms and dot plots.

In histograms and dot plots you can:

- zoom (enlarge or reduce using the mouse) the graphs to display details, for example.
- put a color gradient on the background of the graphs.

In histograms, you can additionally:

- show data points.

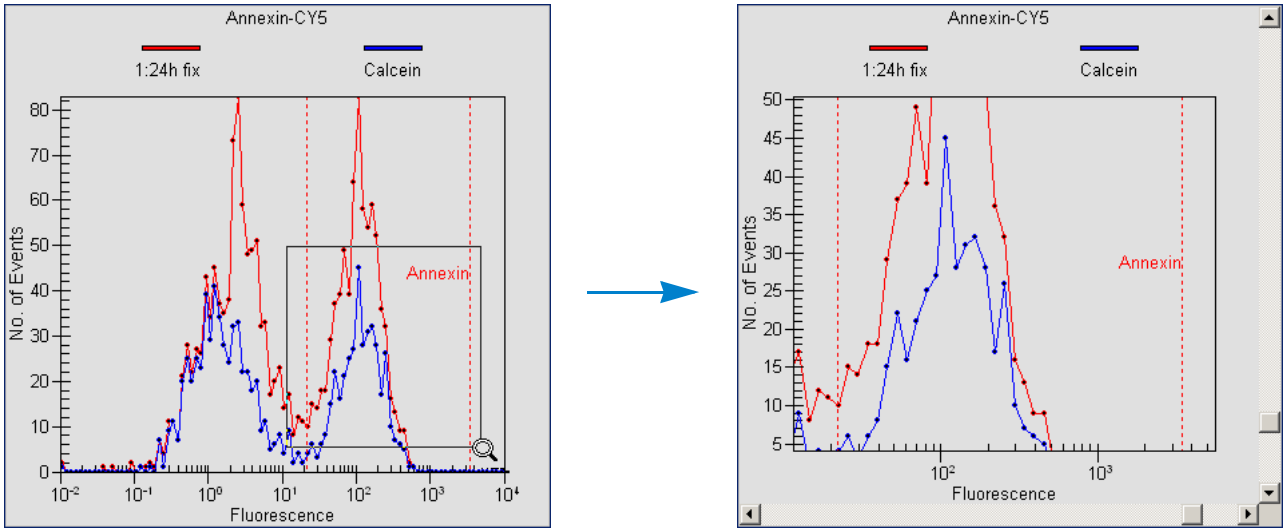
To zoom into a histogram or dot plot:

1 Position the mouse pointer in the histogram/dot plot.

2 Click and hold down the left mouse button.
The mouse pointer changes its shape to a magnifying glass .

3 Drag the mouse.
A rectangle shows the part of the histogram/dot plot to be enlarged.

4 Release the mouse button.



You can perform several zoom steps in a row. When you have zoomed a histogram or dot plot, the *Undo Zoom* and *Undo All* buttons are enabled.

To undo one zoom step:

1 Click the *Undo Zoom* button or double-click in the histogram or dot plot.

To undo all zoom steps:

1 Click the *Undo Zoom All* button.

To display data points in histograms:

1 From the *Histogram* menu, select *Show Data Points*.

All events are shown as bullets.

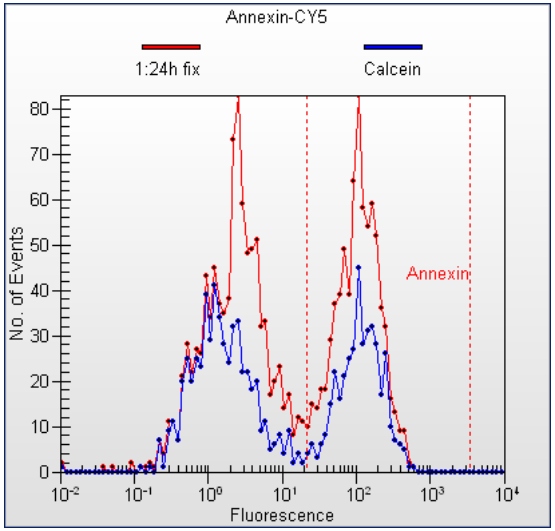
To put a color gradient on the background of a histogram or dot plot:

1 From the *Histogram* or *Dot Plot* menu, select *Gradient*.

– OR –

Click the *Gradient* button the histogram or dot plot toolbar.

A color gradient (gray to white) appears on the background of the graph.



Analyzing and Evaluating the Results of a Flow Cytometric Assay

You can analyze and evaluate result data of flow cytometric assays using either the dot plot or the histogram view. In both views, you can evaluate the detected cells by defining areas of interest.

- *Histograms* show the distribution of events related to the red and blue fluorescence intensity. Gating is used to generate subsets based on markers in one histogram. See "Using Histograms for Evaluation" on page 212 for detailed information.
- *Dot plots* show events as dots in a coordinate system where the blue fluorescence value is related to the red. Regions and gates are used to determine the number of cells with a fluorescence intensity lying in a defined range. See "Using Dot Plots for Evaluation" on page 233 for detailed information.

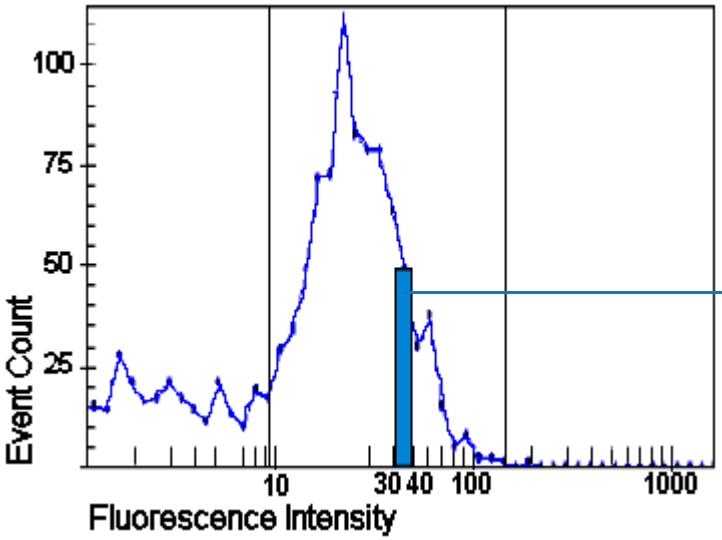
If you use predefined assays, the markers and regions are set at the approximate position where the events are expected. Refer to "Evaluating Antibody Staining, Apoptosis, and GFP Assays" on page 242 for information on how to evaluate the predefined assays.

TIP

You can analyze and evaluate results while a chip run is still in progress.

Using Histograms for Evaluation

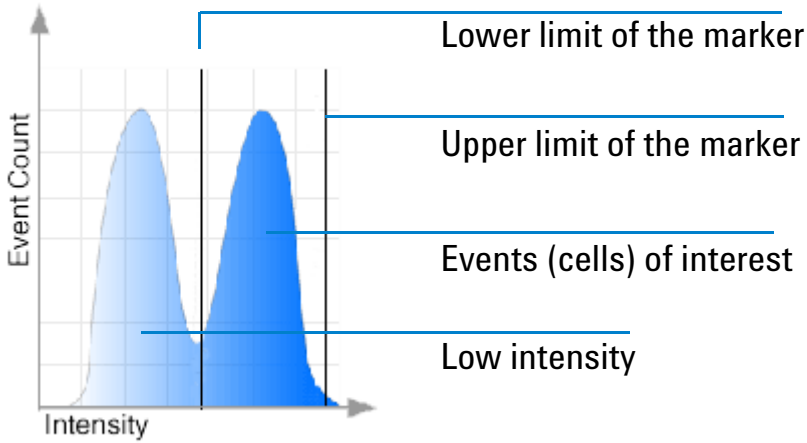
Histograms are graphical representations of the measurement results, where the number of events (cells) is mapped to the Y axis and their fluorescence values to the X axis. The resulting curves show the frequency distribution of the events in relation to their fluorescence intensity values, as shown in the following image. In real histograms, the bin is reduced to a dot (data point).



The histograms can be evaluated statistically with markers that allow you to define ranges of fluorescence intensity values. One histogram can be used to represent a range of fluorescence values to define a subset of events. Only cells with a fluorescence value within this range are displayed in the second histogram. This method is called gating.

Markers

Markers are used to define a range of fluorescence intensity values in a histogram. The upper and lower limits of the range are displayed as vertical lines, as shown in the following image.



The numerical values for each defined marker are displayed in a separate row in the result table. One marker is used as a gate for the second histogram, to define a subset of events.

In predefined assays, the markers are set by default and you only need to adjust their position. If you want to define your own assays, select the *Generic* assay for acquisition, where you can define your own markers and/or regions together with gates.

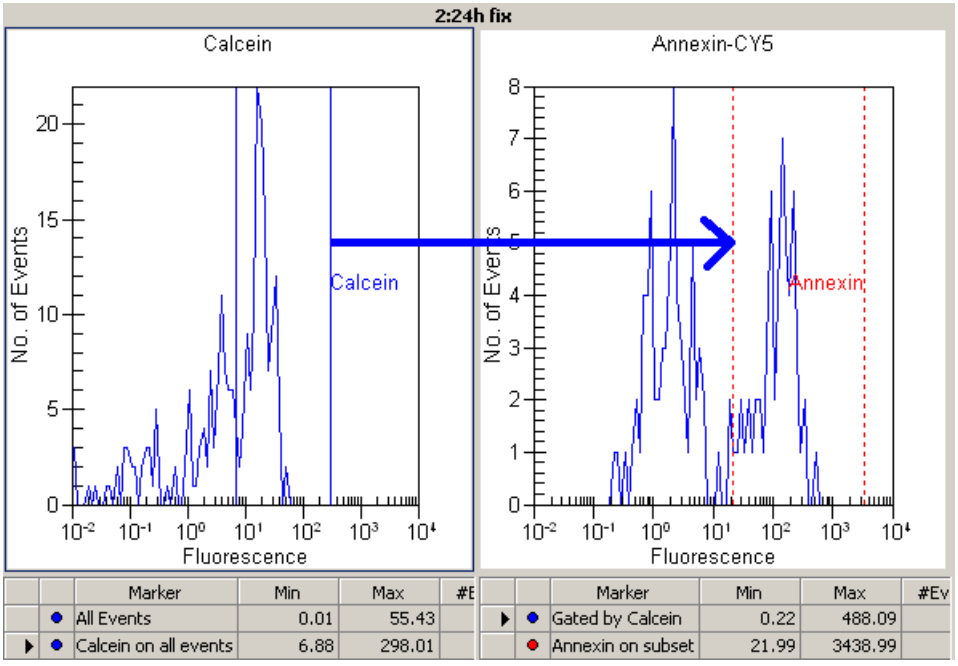
If you use several markers within one histogram, only one of them can be used for gating. The other markers can only be used to evaluate regions in the histogram they cover. The values belonging to these markers are also displayed in the result table.

Gating

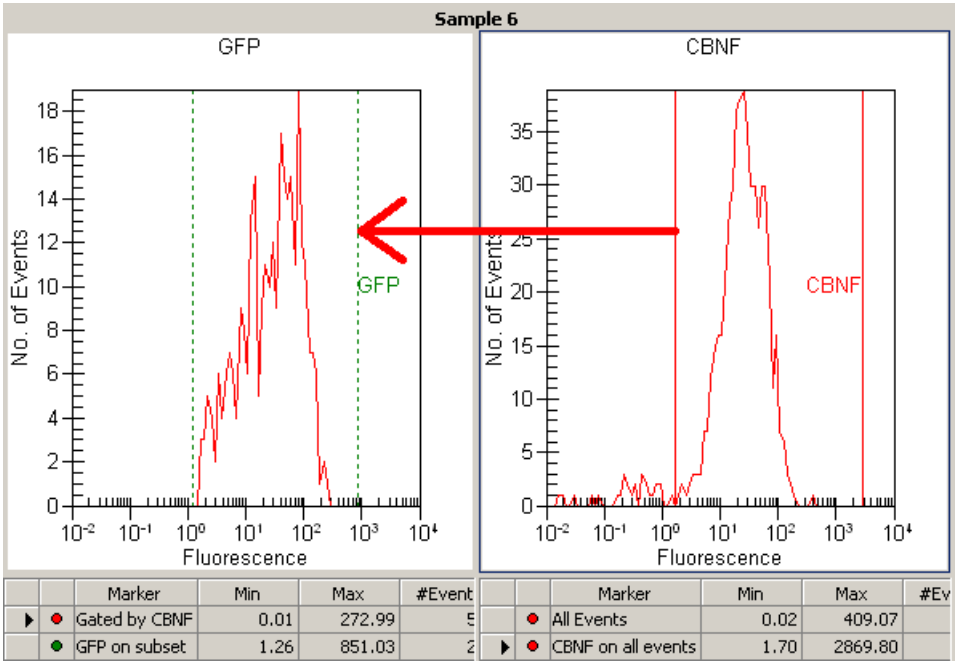
Gating is used to restrict the number of events that are evaluated by gating out (filtering) events that do not have the fluorescence values set by a marker. For example, by gating on a blue marker, you can exclude all events with low blue fluorescence (allowing you, for example, to gate out dead cells, unbound dye and debris). Only events with blue fluorescence values within the marker range are evaluated. Thereby, you can exclude any dead cells, and evaluate only the living cells for another property.

The gating direction defines the reference histogram:

- Gating from *blue to red* uses the blue histogram to define the subset by a marker (*Apoptosis* and *Antibody Staining* assays).



- Gating from *red to blue* uses the red histogram to define the subset by a marker (*GFP* assay).

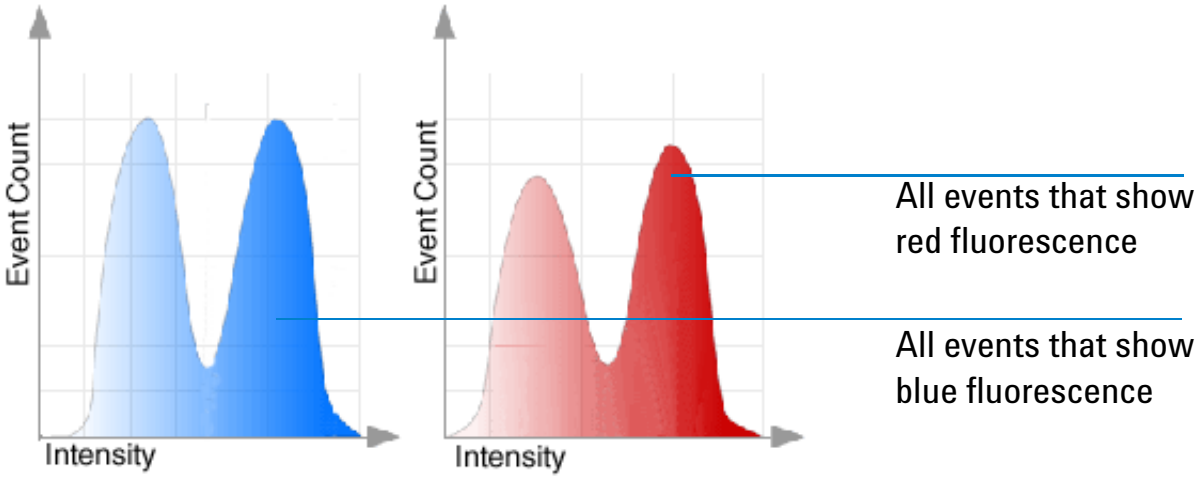


NOTE

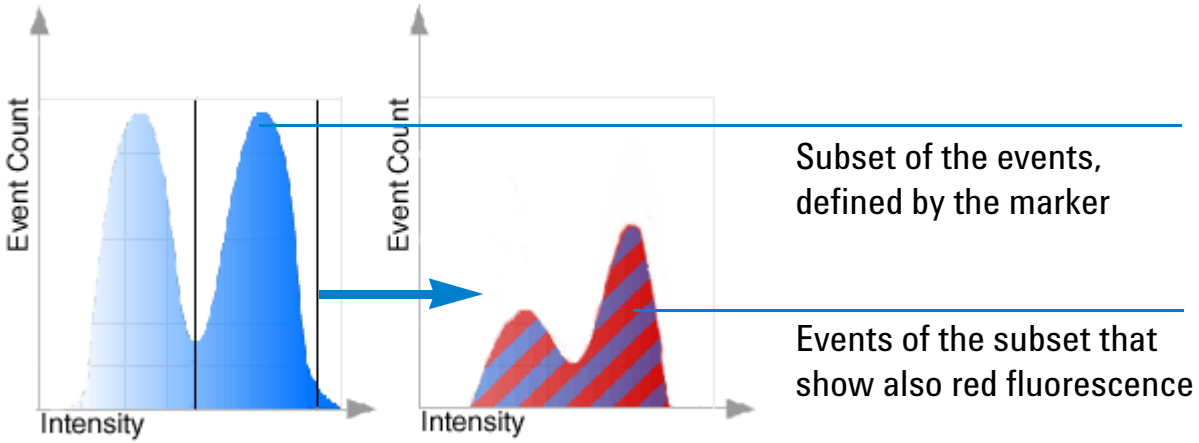
Predefined assays have a fixed gating direction, while assays of type *Generic* have a variable gating direction.

The following figures illustrate gating from blue to red.

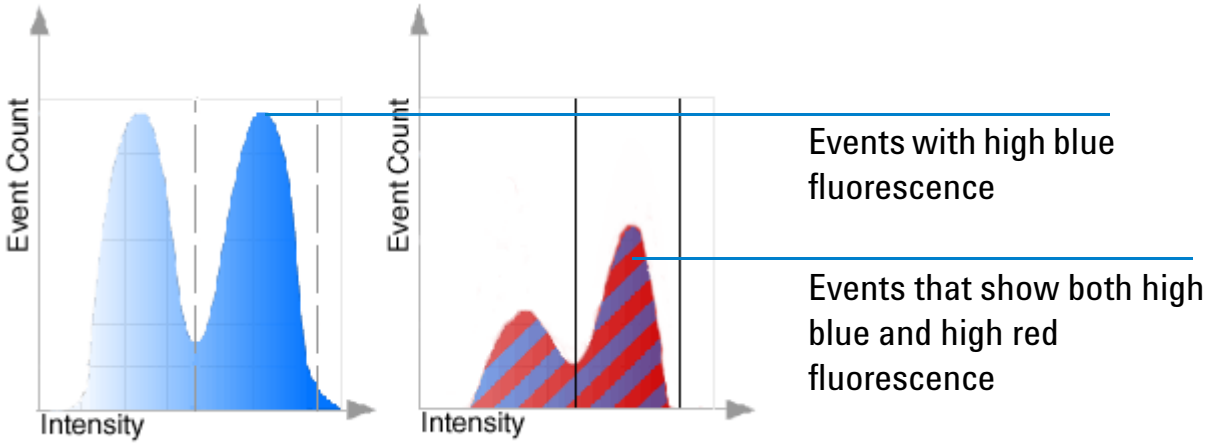
The two histograms display all measured events in the blue histogram and in the red histogram without gating. In this case, you cannot see which cells fluoresce only in the blue and which fluoresce only in the red.



By setting a marker on the blue histogram, you can define the blue fluorescence range that must be met for a cell to be considered for the red histogram. You use the gating on the blue histogram to define a subset for the red histogram.



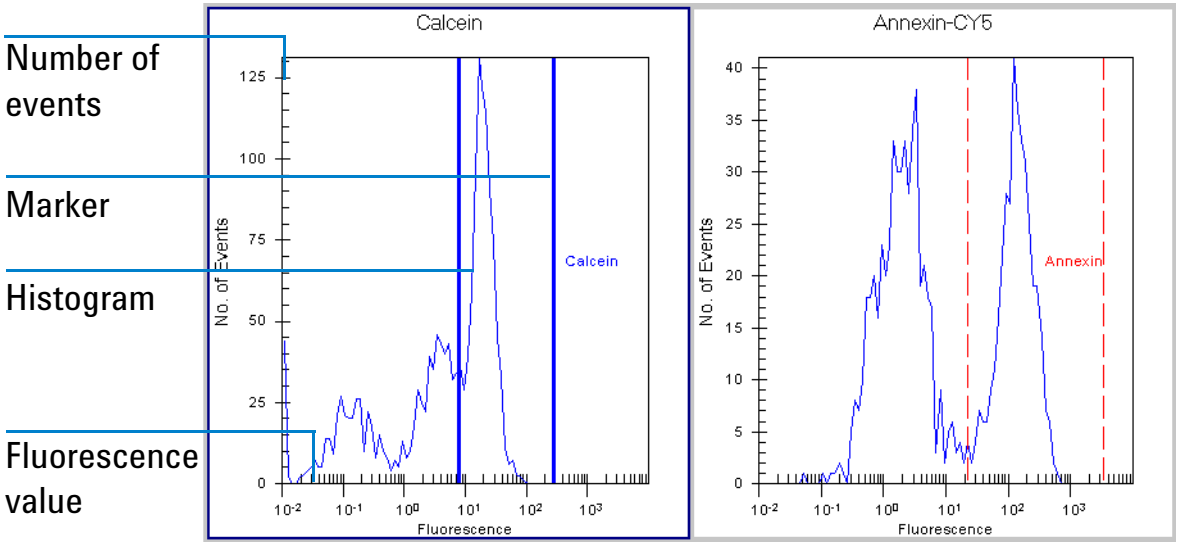
The red histogram displays now only cells with blue and red fluorescence within the marker. To evaluate this subset, you can set a marker in the red histogram. This second marker filters out all cells that do not have fluorescence in this range.



The result table (see also "Displaying the Results of Histogram Evaluations" on page 230) of the gated histogram (here the red one) shows the values numerically:

- The *% total* value shows the number of events that have both high blue and high red fluorescence, in relation to all measured events.
- The *% of gated* value shows the number of events that have high blue and high red fluorescence in relation to the blue (or red) events.

The following image shows two histograms with a gating direction from blue to red (left to right) of an apoptosis assay. The blue histogram shows calcein fluorescence, which indicates living or dead cells (high fluorescence value means living cells). The red histogram shows the subpopulation of living cells with annexin V fluorescence indicating apoptosis (high fluorescence value means the cell is apoptotic). As a result you can see a subset of living, apoptotic cells.



How to Insert a Marker in a Histogram

A marker is shown as two vertical lines that define a region of fluorescence values. It is used to select a subset of events according to this fluorescence region.

NOTE

You can insert markers only in *generic* assays.

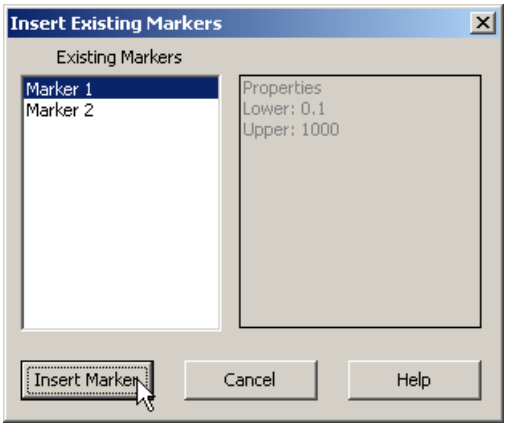
To add a new marker:

1 In the toolbar of the *Histogram* tab, click the *Insert Marker* button .

A marker is added to the selected histogram window.

To insert an existing marker:

1 Click the *Insert Existing Marker* button to open the *Insert Existing Markers* window.



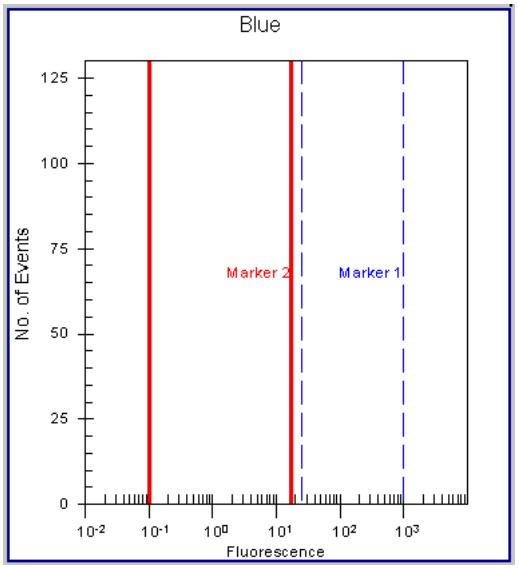
2 Select a marker in the list and click *Insert Marker*.

The marker lines are added at the defined positions. The label identifies the marker.

You can remove markers that you do not need any more:

1 Click one of the vertical lines in the histogram to select the marker.

The lines of the selected marker are displayed bold.



TIP

You can also click the corresponding row in the result table to select the marker.

2 Click the *Delete Marker* button to remove the marker.

How to Configure Markers

You can change the color, name, and the upper and lower limits of the marker:

1 Double-click the desired marker.

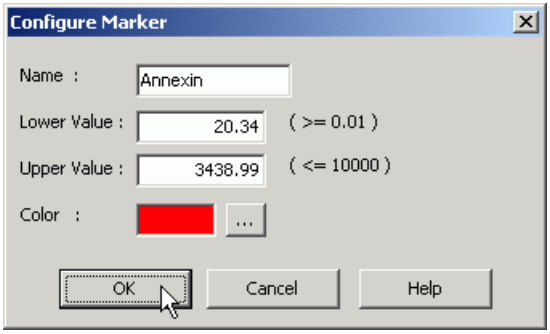
– OR –

Right-click the corresponding row in the result table and select *Configure Marker* from the context menu.

– OR –

Select the marker and click the *Configure Marker* button in the toolbar.

The *Configure Marker* dialog box appears.



2 Enter a name for the marker, for example, the used dye (it is advisable to use names that identify the marker).

3 Enter a *Lower Value* (left vertical line).

4 Enter an *Upper Value* (right vertical line).

NOTE

The lower and upper values must be within the range of 0.01 – 10000 relative fluorescence units.

5 Click the *Color* button to open the *Color* dialog box and select a color.

6 Click *OK*.

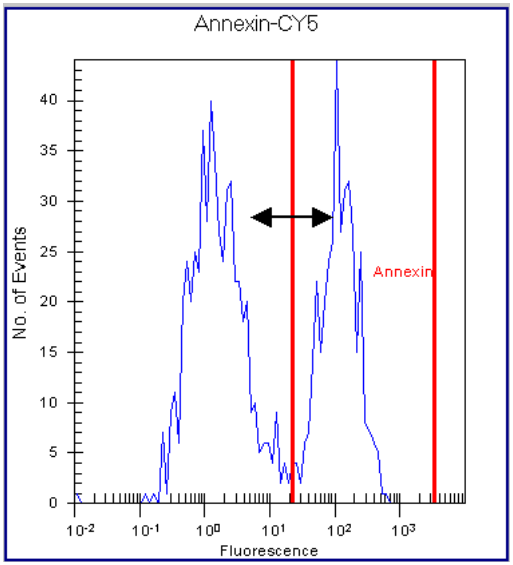
How to Move the Upper and Lower Limits of Markers

You can change the position of both marker lines by dragging them with the mouse:

1 Position the mouse pointer on a marker line.

The mouse pointer changes its shape to a hand.

2 Drag the line to the desired position.



3 Release the mouse button.

4 Repeat these steps for the other marker line, if necessary.

NOTE

You can change the marker limits also by entering fluorescence values in the *Configure Marker* dialog box (see "How to Configure Markers" on page 222).

How to Copy Markers to All Histograms

Once a marker is defined, you can copy it in the histograms of all samples (generic assays only):

1 Select the marker in the histogram or in the result table.

The *Insert the selected marker into all histograms* button is now enabled.

2 Click this button.

The *Copy Marker* dialog box appears. This dialog box asks you whether or not you want to use the marker as reference.

3 Click *Yes* to use this marker as reference. The marker will be inserted in all other histograms of the blue or red channel. When the properties of this marker are changed, the changes will be applied to all samples.

– OR –

Click *No.* The marker will be inserted in all other histograms of the blue or red channel. When the properties of this marker are changed, the changes are only applied to the current sample.

How to Set the Gating Direction (Generic assay only)

You can use *one* marker to define the gating direction. In other words, you define whether red or blue fluorescence is used as a gate to define a subset in the other histogram. This also depends on the dyes that you have used for staining.

You can set both gating directions: either from the blue histogram to the red histogram or from red to blue.

To set the gating direction:

1 Select the marker in the red or blue histogram you want to use as a gate for the other histogram.

  The corresponding gating button in the toolbar is now enabled.

2 Click or to set the gating direction.

  – OR –

  Right-click the marker in the histogram or in the result table, and select *Gate in Red/Blue histogram* from the context menu.

The gating direction is displayed in the *Information Bar*.

If the gating direction is already set, you first have to remove the existing gating.

To remove gating:

1 Click the *Remove Gate* button .

  The gating is removed and the corresponding gating button is enabled.

NOTE

To change the gating direction in non-generic assays, you first have to change the assay to generic. To achieve this, use the *Import Setpoints* button on the *Assay Properties* tab (refer to "Importing Data" on page 271).

How to Overlay Histograms

You can compare samples by overlaying their gated histograms. This is useful, for example, if you want to see the progress of a reaction or if one sample is used as reference.

Overlaying histograms might also be helpful for adjusting the marker position. You can overlay all measured samples. Both red and blue histograms are overlaid.

NOTE

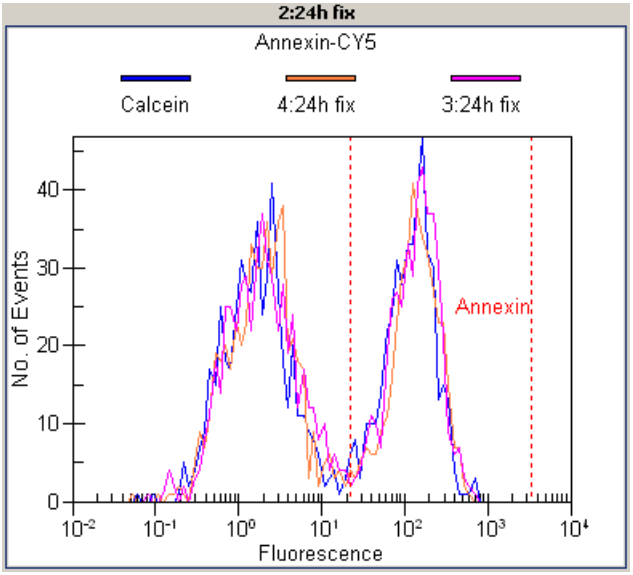
You can configure the colors of the overlaid histograms and adjust the scale graduation as described in "How to Set Signal Colors for Overlaid Histograms" on page 229.

To overlay histograms:

1. Select the main sample and display the *Histogram* tab.
2. Click the *Overlaid Samples*

button to open a drop-down list.
3. Click the sample that you want to use as overlay.

   The histogram curve of the selected sample appears in the histogram view, the corresponding entry in the drop-down list is marked with a check, and a color legend appears above the graph.
4. Repeat steps 2 and 3 to overlay further histograms.



To overlay all samples:

1 Click the *Overlaid Samples* Overlaid Samples button to open a drop-down list.

2 Select *All Samples* to overlay the histogram curves of all samples.

To remove histograms from the overlay:

1 Select the sample that contains the overlaid histograms.

2 Click the *Overlaid Sample* button Overlaid Samples to open the drop-down list.

3 Click the sample that you want to be removed.

– OR –

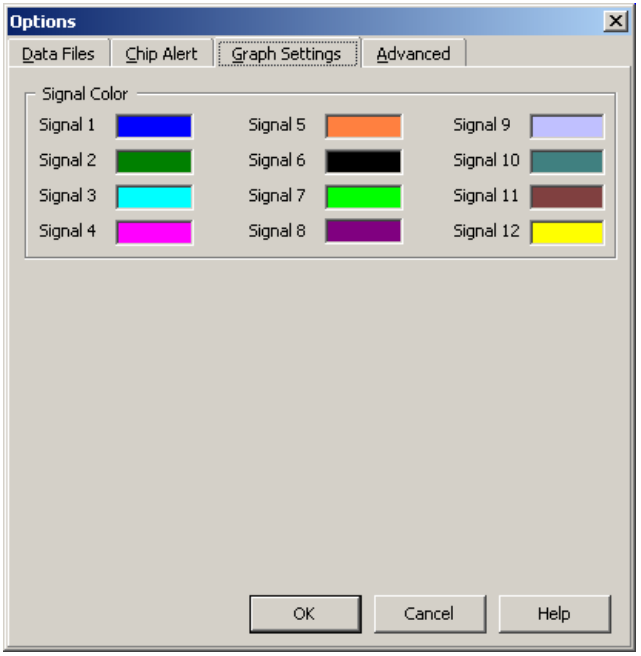
Click *No Overlay* to remove all overlaid curves from the histogram.

How to Set Signal Colors for Overlaid Histograms

You can use the *Graph Settings* tab in the *Options* dialog box to configure the signal colors (colors of curves in histograms):

1 Select *Tools* > *Options*.

2 Click the *Graph Settings* tab to bring it to the front.



To configure the signal color:

1 Click the colored square corresponding to the signal.

The *Color* dialog box appears.

2 Select a color for the signal and assign it by clicking *OK*.

Displaying the Results of Histogram Evaluations

The calculated results are displayed in result tables, one table below each histogram. Markers, gates, several statistical values, and the %-values of events are shown in the result tables.

		Marker	Min	Max	#Events	%Total	% of gated	Mean	StdDev	CV%	GMean
▶	●	All Events	0.02	51.42	1606	100	-	8.07	7.13	88.37	4.51
	●	Gated by Calcein	0.04	51.42	1418	88.30	-	8.46	7.32	86.47	4.72
	●	CD3-APC on all events	1.18	4085.88	1356	84.40	-	9.48	6.89	72.75	7.27
	●	CD3-APC on subset	1.18	4085.88	1195	74.40	84.30	9.96	7.02	70.44	7.73

Each marker you insert in the histogram gets its own row. Note that you can only use one marker for gating. The additional markers can be used to evaluate different parts of the histogram statistically.

If the option *Hide superset curve* is disabled in the setpoint explorer, two additional rows are displayed in the gated histogram's result table. The superset curve shows a histogram of all measured events; the gate is not considered.

You can export the table data for further evaluation in other applications. See "Exporting Data" on page 277.

NOTE

The layout of the result table can be configured (see "Configuring Tables" on page 293). Not all of the values listed below may therefore be visible.

The content of the result tables depends on the gating direction. The histogram that is used for gating can display the following results:

Marker	All events – this row shows the data for all measured events, for example, for all living and dead cells. The following rows show the data for the subset of cells defined by the inserted marker. If you use a predefined assay, the entry can be "Calcein on all events", for example.
Min	Minimum fluorescence value of the corresponding marker.
Max	Maximum fluorescence value of the corresponding marker.
Events	Number of events covered by the marker. For the histogram you use for gating, the number of all detected events is displayed in the row "All Events".
% total	% of selected events in relation to the total number of events. The row "All Events" shows 100%.
% of gated	% of events covered by the marker in the gated histogram. Shows no value for the gating histogram.
Mean	Mean fluorescence value of the events inside the marker.
StdDev	Standard deviation to the mean value.
%CV	Coefficient of variation.
GMean	Geometric mean.

The histogram that displays the gated data can show the following data:

Marker	All events – this row shows the data for all events that pass the gate. The following rows show the data for all events covered by the inserted marker. If you use a predefined assay, the entry can be "Annexin V on subset", for example.
Min	Minimum fluorescence value of the corresponding marker.
Max	Maximum fluorescence value of the corresponding marker.
Events	Number of events covered by the marker. For the histogram you use for gating, the number of all detected events is displayed in the row "All events".
% total	% of selected events in relation to the total number of events. The marker used for gating is 100%, the table of the gated histogram shows the value of the subset.
% of gated	% of the gated events in relation to the total number of events. These are the events that have passed the gate and are covered by the marker of the histogram, for example, by annexin V.
Mean	Mean fluorescence value of the events inside the marker
StdDev	Standard deviation to the mean value.
%CV	Coefficient of variation.
GMean	Geometric mean.

Using Dot Plots for Evaluation

On the *Dot Plot* tab, cells are displayed as dots, where their red fluorescence intensity is mapped on the Y axis and their blue fluorescence intensity is mapped on the X axis.

NOTE

The lower left region of the dot plot area may show no events, because of the threshold for event detection. Dots are only displayed if their fluorescence intensity exceeds a minimum limit. The limits are specified in the assay—separately for red and blue fluorescence.

To evaluate the dot plots, you can add *regions*. Regions are rectangles that can be changed in size and position until they include a specific event subset. As a result you get the number of cells included in the region related to the total number of cells.

NOTE

You can add/remove regions and gates only in *Generic* assays.

Additionally, you can insert a horizontal or a vertical gate for *one* region. This is useful for counting all cells that have fluorescence intensities within the horizontal or vertical borders of the region. In predefined assays, the vertical side of a region corresponds to the marker of the blue histogram, the horizontal side to the red one (see "Using Histograms for Evaluation" on page 212). The gate is always displayed and corresponds to the range of the marker that is used for gating. If you move a marker in a histogram, the region and gate are automatically updated. If you change a region or gate, the marker is also updated. Statistics are displayed in the result table below the dot plot.

How to Add Regions to Dot Plots (Generic Assay only)

You can draw regions in dot plots of generic assays. If there are regions already defined in other samples, you can copy these regions in the dot plot of the current sample.

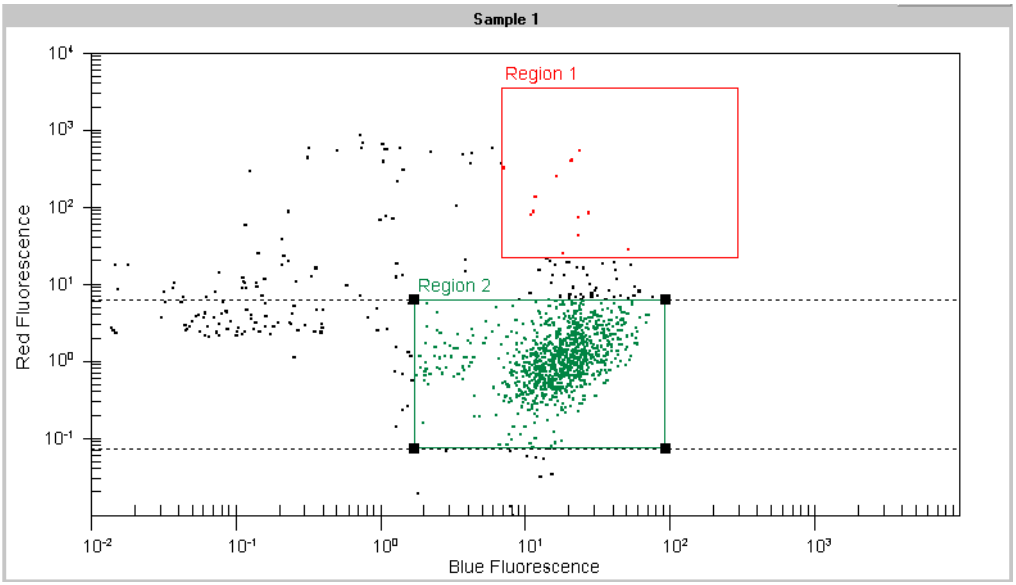
To draw a new region:

1 Click the *Insert Region* button in the toolbar.

The mouse pointer changes its shape to a crosshair.

2 Draw a rectangle into the dot plot.

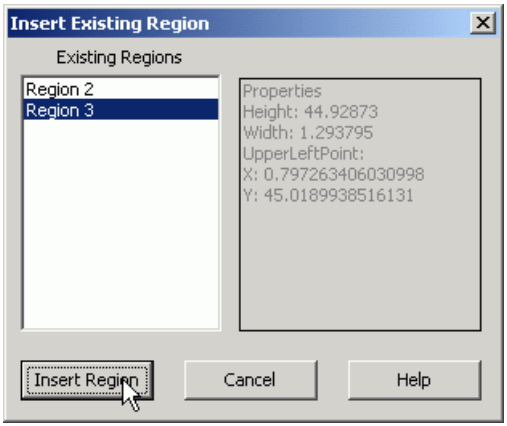
New regions are automatically named "Region x", where x is an auto-incremented number. By default, the border color of new regions is black. To make it easier to differentiate between regions, you can change their border color (see "How to Configure Regions" on page 236).



To insert an existing region:

1 Select the sample where you want to insert an existing region from another sample and click *Insert existing region* .

The *Insert Region* dialog box appears.



2 Select the region that you want to insert and click *Insert Region*.

The region is inserted at its predefined position.

To remove a region:

1 Click the region border to select the region that you want to remove.

The selected region is highlighted. and the *Delete Regions* button is enabled.

2 Click this button.

The region disappears from the dot plot.

How to Configure Regions

You can change the color of the region border, edit the region's name, and define the position and size of the region.

To configure a region:

1 Double-click the border of the region that you want to configure.

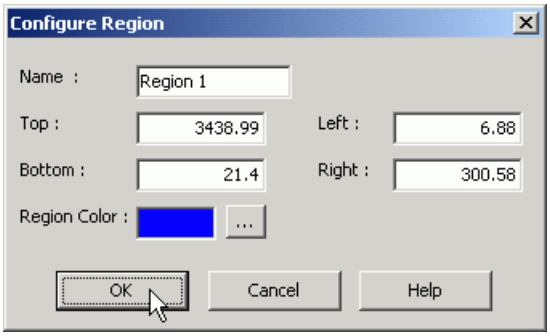
– OR –

Right-click the corresponding row in the result table and select *Configure Region* from the context menu.

– OR –

Click the region border to select the region, and click the *Configure Region* button in the toolbar.

The *Configure Region* dialog box appears.



2 Enter a *Name* for the region.

It is advisable to use an easy-to-understand name.

3 Enter fluorescence values for the left, right, bottom, and top side of the rectangle to define position and size of the region.

   These values correspond to the upper and lower marker limits of the blue and red histograms.

4 Click the ... button next to the color square to open the *Color* dialog box, and select a color for the region border.

5 Click *OK*.

To color dots inside regions:

1 Click the *Color Dots* button in the toolbar.

All dots inside the regions now have the same color as the region border. In case of overlapping regions, dots are colored with the color of the last added, re-positioned or resized region.

How to Change Position and Size of a Region

You can change the size and position of regions to restrict the number of included events. You can work graphically with the mouse or enter the values in the *Configure Region* dialog box.

To move a region:

1 While pressing the Shift key, click the region border, and drag the region to the new position.

2 Release the mouse button.

To change size and position with the mouse:

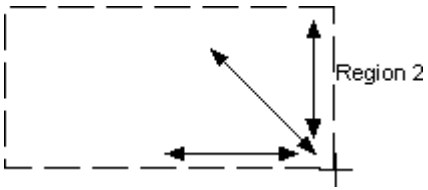
1 Click the region border to select the region.

2 Position the mouse pointer on any corner of the selected region.

The mouse pointer changes its shape to a double arrow.

3 Click and drag the border to the new size.

Upon moving, the mouse pointer changes its shape to a crosshair and the borders of the region appear as dashed lines.



4 Release the mouse button.

To change size and position numerically:

1 Double-click the region to open the *Configure Region* dialog box.

2 Enter fluorescence values for the left, right, bottom, and top side of the rectangle to define position and size of the region.

These values correspond to the upper and lower marker limits of the blue and red histograms.

3 Click *OK*.

How to Insert a Region in All Dot Plots

If you have defined a region for one sample, you can copy it to the other samples of the assay.

To insert a region in all dot plots:

1 Left-click the region border to select the region that you want to use as source.

The *Insert region into all dot plots* button is enabled.

2 Click this button

The *Copy Region* dialog box appears, which asks whether or not the source region should be used as reference. The region will be inserted in the dot plots of all other samples. When you change the properties of the region, all copies of the region will also be changed.

3 Click *Yes* to define the source region as reference.

– OR –

Click *No* to create new regions that are not "connected". The region will be inserted in the dot plots of all other samples. When the properties of the region are changed, the changes affect only the selected sample.

The region is copied to all samples of the assay.

How to Work with Gates in Dot Plots

You can insert gates only in *generic* assays. For predefined assays, the gate is already defined.

Before you can insert a gate, you have to draw a region (see "How to Add Regions to Dot Plots (Generic Assay only)" on page 234). If a gate is already set, you first have to remove the existing gate.

To add a gate to a region:

1. Left-click the region border to select the region to which you want to add the gate.

   The gating buttons in the toolbar are enabled.

2. Click the *Horizontal Gate* button or the *Vertical Gate* button to set a gate along the horizontal or the vertical borders of the selected region.

   In the result table, a value appears in the *% of gated* column.

If the gating direction is already set, you first have to remove the existing gate:

1. Left-click the region border to select the region.

   If a gate already exists, the *Remove Gate* button is enabled.

2. Click this button.

The gate is removed and the gating buttons are enabled.

Displaying the Results of Regions

The measurement results and calculations for regions are displayed in the result table below the dot plot. In predefined assays, only one region is available, while for generic assays, dot plots can have as many regions as you like.

The following values are displayed:

Region	The default region *All Events* is always displayed in the first row and shows the values for all detected events. For each further region (see "How to Add Regions to Dot Plots (Generic Assay only)" on page 234), a row is added to the table.
XMean	Mean fluorescence values in x direction.
YMean	Mean fluorescence values in y direction.
#Events	Number of events for each region added to the dot plot.
% Total	Percentage of events for each region added to the dot plot.
% of gated	Percentage of the gated events in the region.
StdDevX	Standard deviation to the mean fluorescence value in x direction.
StdDevY	Standard deviation to the mean fluorescence value in y direction.
CV%X	Coefficient of variation of the x values.
CV%Y	Coefficient of variation of the y values.
X GMean	Geometric mean of the x values.
Y GMean	Geometric mean of the y values.

Evaluating Antibody Staining, Apoptosis, and GFP Assays

With the 2100 expert software, several predefined assays are supplied. You should only use each assay for the specific experiment for which it was developed. For example, you have to use the read dye for detection of apoptosis (calcein and Cy5, for example):

- "Evaluating Antibody Staining Assays" on page 243.
- "Evaluating Apoptosis Assays" on page 247.
- "Evaluating GFP Assays" on page 253.

Evaluating Antibody Staining Assays

Antibody staining lets you measure protein expression on the surface or inside a cell by means of specific antibodies. Either the primary antibody itself is conjugated with a dye or you must use a labeled secondary antibody that recognizes the primary antibody. When you measure the fluorescence of the cells, you can compare the relative expression of protein in individual cells and use this information for population analysis. Typically, you can use a red dye such as APC (Allophycocyanin) or Cy5 to measure antibody presence.

You can use a blue dye like calcein to detect whether or not the cells are living, or like SYTO 16 to stain the nucleic acids of all cells. For detailed information, refer to the application note *Detecting Cell Surface and Intracellular Proteins with the Agilent 2100 Bioanalyzer by Antibody Staining*.

For a detailed description on how to evaluate the results using markers and regions, refer to "Using Histograms for Evaluation" on page 212, and to "Using Dot Plots for Evaluation" on page 233.

Gating direction

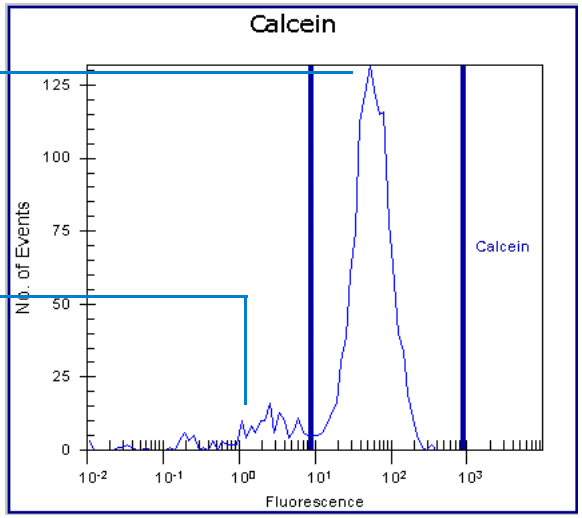
The gating direction is from blue fluorescence to red fluorescence. Depending on the dye you use, you should use all cells (nucleic acid dye) or only living cells (calcein living dyes) for gating.

Histogram Evaluation

The blue histogram is used for gating. High fluorescence in the blue histogram means that the cells are living (if a life-indicating dye is used). Low fluorescence means the cells are dead. If you use a nucleic acid dye, you cannot distinguish between living and dead cells, you can only count all measured cells. See the following image as an example:

High fluorescence value indicates living cells

Low fluorescence value indicates dead or non-healthy cells



The values are displayed in the result table below the blue histogram:

All measured events

All events in relation to the blue marker (here calcein)

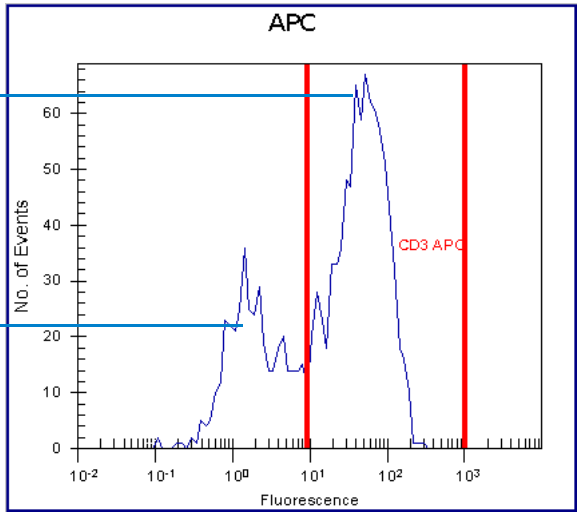
		Marker	# Events	% total	% of gated
▶	●	All Events	1565	100	-
	●	Calcein on all events	1436	91.8	-

Living cells related to all measured cells (high calcein fluorescence)

When using the calcein marker in the blue histogram for gating, only living cells are considered for building the histogram of the red dye. High red fluorescence values indicate living cells with bound antibodies, low red fluorescence values living cells without bound antibodies. See the following example.

High fluorescence values indicate binding of labeled antibodies

Low fluorescence values indicate low binding of labeled antibodies



The values are displayed in the result table below the red histogram:

Percentage of the living cells

Percentage of all cells with high red fluorescence selected by the red marker

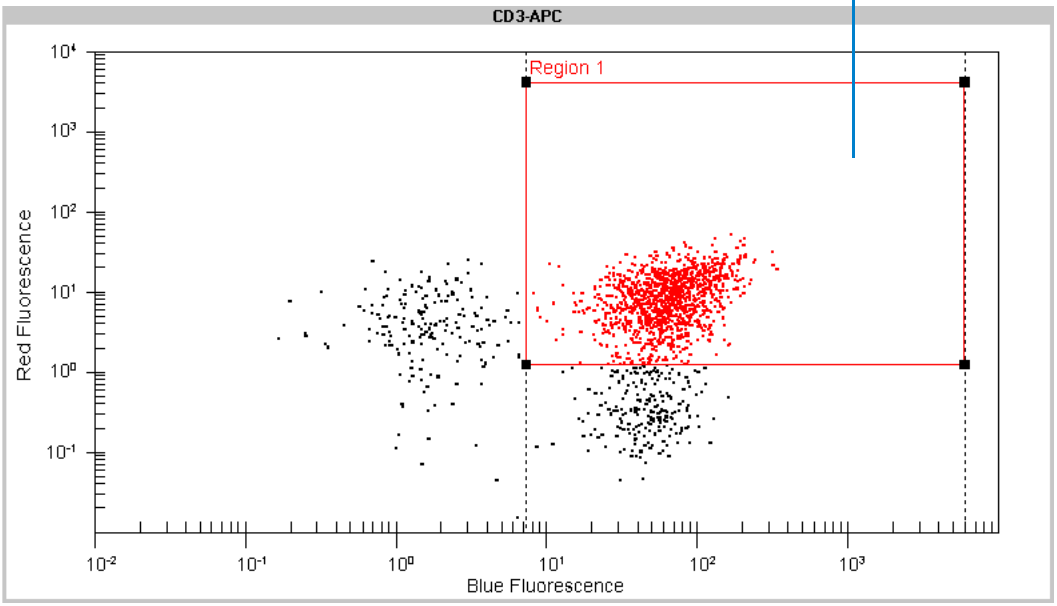
		Marker	# Events	% total	% of gated
▶	●	Gated by Calcein	1436	91.8	-
	●	CD3-APC on subset	25	1.6	1.7

Amount of living cells with high red fluorescence in relation to the amount of living cells

Dot plot evaluation

If you switch to the *Dot Plot* tab, one region is displayed in the dot plot. The red fluorescence values of the region are related to the marker in the red histogram, the blue fluorescence values to the marker in the blue histogram. As in the histogram evaluation, high blue fluorescence and high red fluorescence mean living cells with bound antibodies. See the following example.

Cumulation of high blue and high red fluorescence indicates strong binding of labeled antibodies in living cells



The results of the dot plot evaluation are numerically displayed in the result table:

Events covered by the region

All measured events

		Region	# Events	% total	% of gated
	●	All Events	1606	100	-
▶	●	Region 1	1193	74.3	84.1

Amount of living cells in relation to all measured cells

Amount of living cells with high antibody binding in relation to all living cells

Evaluating Apoptosis Assays

The apoptosis assay can be used to examine how many apoptotic cells are within a living cell population. Dead (or necrotic) cells can be excluded from the evaluation.

For a detailed description on how to evaluate the results using histograms and regions, refer to "Using Histograms for Evaluation" on page 212 and "Using Dot Plots for Evaluation" on page 233.

Living or dead cells

In most cases, you want to know whether cells are dead or alive at a specific time. For this, you can use calcein-AM as living cell dye, for example. This dye accumulates in intact cells, whereas it will leak out of damaged cells. Once inside the cells, the colorless AM ester is cleaved by esterases, resulting in the formation of the highly fluorescent calcein. The number of events resulting from a calcein-related staining thus gives you the

number of living cells in a sample. For detailed information, refer to the application note *Apoptosis Detection by Annexin V and Active Caspase 3 with the Agilent 2100 Bioanalyzer*.

Apoptotic cells

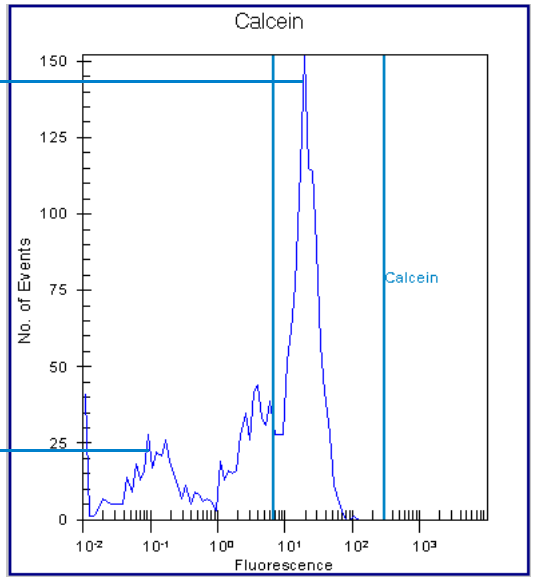
In apoptotic cells, phosphatidylserine is no longer confined to the inner leaflet of the plasma membrane bilayer. Phosphatidylserine becomes accessible on the outer surface of the cell membrane and can be bound with high affinity by the protein annexin V, which can be labeled with biotin or dyes such as Cy5.

Gating direction

The gating direction is from blue fluorescence (living cells) to red fluorescence (annexin).

Histogram evaluation

The two histograms displaying the results of the assay are related to calcein (blue fluorescence) and annexin V (red fluorescence). High fluorescence values in the blue histogram indicate living cells, low values correspond to dead cells. See the following image as an example.



High fluorescence value indicates living cells

Low fluorescence value indicates dead cells

The values are displayed in the result table, each histogram has its own table:

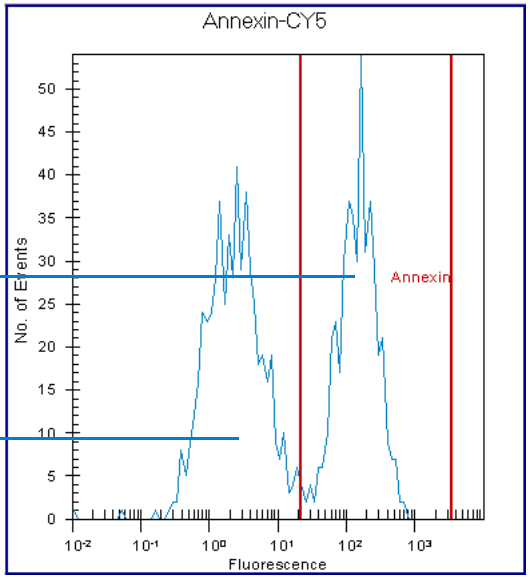
All measured events

All events in relation to the blue marker (here calcein)

	Marker	# Events	% total	% of gated
▶	All Events	1736	100	-
	Calcein on all events	998	57.5	-

Living cells in relation to all measured cells (high calcein fluorescence)

When using the calcein marker in the blue histogram for gating, only the living cells are considered for building the red histogram. High red fluorescence values indicate living, apoptotic cells, low red fluorescence values indicate living, non-apoptotic cells. See the following example.



High fluorescence value indicates living apoptotic cells

Low fluorescence value indicates living non-apoptotic cells

Amount of the living cells in relation to all measured events

Percentage of all cells with high red fluorescence selected by the red marker

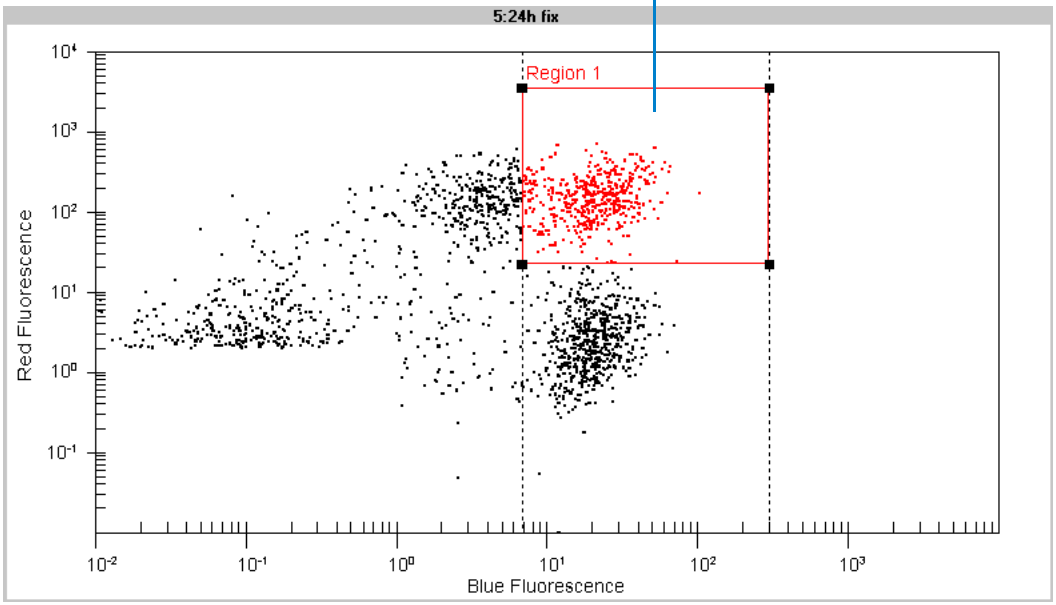
	Marker	# Events	% total	% of gated
●	Gated by Calcein	998	57.5	-
▶ ●	Annexin on subset	444	25.6	44.5

Amount of living cells with high red fluorescence in relation to the amount of living cells

Dot plot evaluation

If you switch to the *Dot Plot* tab, one region is displayed in the dot plot. The red fluorescence values of the region are related to the marker in the red histogram, the blue fluorescence values to the marker in the blue histogram. As in the histogram evaluation, high blue fluorescence and high red fluorescence represent living cells with annexin V binding. See the following example.

Cumulation of high blue and high red fluorescence indicate living apoptotic cells



The results of the dot plot evaluation are displayed in the result table.

All measured events

Events covered by the region

		Region	# Events	% total	% of gated
	●	All Events	1736	100	-
▶	●	Region 1	444	25.6	44.5

Amount of living cells with high red fluorescence in relation to the amount of all cells

Amount of living cells with high red fluorescence in relation to the amount of living cells

Evaluating GFP Assays

With GFP (Green Fluorescent Protein) assays, the fluorescent substance is not a dye, but a protein. Cells can be transfected with a target gene together with the GFP-producing gene. Transfected cells produce the fluorescent protein, which can be detected. The fluorescence shows you the success of the transfection experiment. For detailed information on GFP assays, refer to the application note *Monitoring transfection efficiency by green fluorescent protein (GFP) detection with the Agilent 2100 Bioanalyzer*.

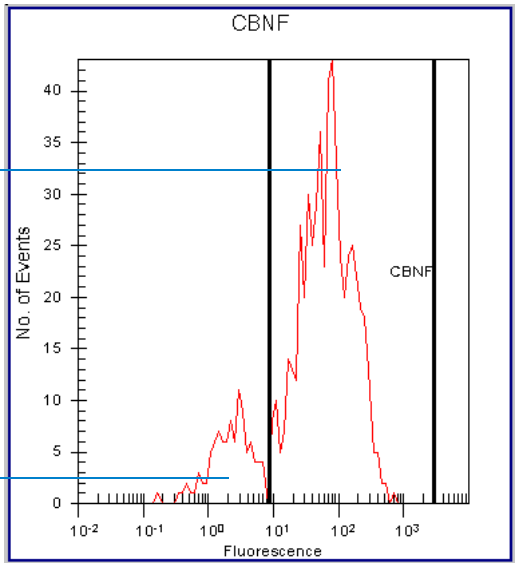
For a detailed description on how to evaluate the results using histograms and regions, refer to "Using Histograms for Evaluation" on page 212 and "Using Dot Plots for Evaluation" on page 233.

Gating direction

The GFP has a green fluorescence (absorption in the blue). Because the reference dye (CBNF) fluoresces in the red, the gating direction is from red to blue. CBNF stains living cells, so you can detect living, GFP-positive cells.

Histogram evaluation

The two histograms displaying the results of the assay are related to CBNF (red fluorescence) and GFP (blue fluorescence). High fluorescence values in the red histogram indicate a staining with CBNF, which is associated with living cells. See the following image as example.



High fluorescence value is associated with living cells

Low fluorescence value indicates dead cells

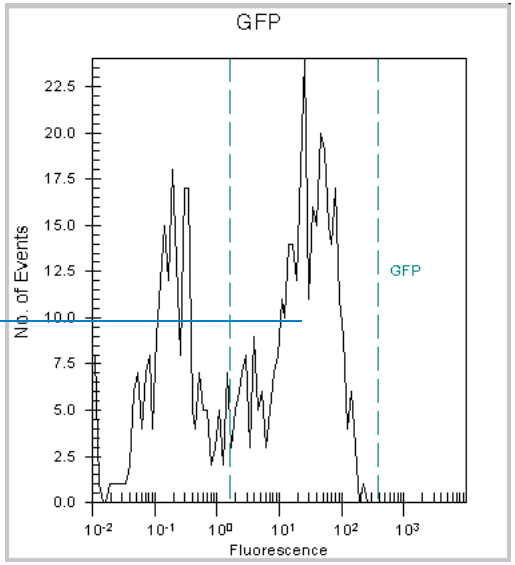
The values are displayed in the result table, each histogram has its own table.

All events related to the red marker (here CBNF)

All measured events

		Marker	% of gated	% total	# Events
▶	●	All Events	-	100	658
	●	CBNF on all events	-	84.7	557

After gating by using the red histogram, in the blue histogram only CBNF-stained cells are displayed. High blue fluorescence values indicate GFP-producing cells. See the following example.



High fluorescence value indicates GFP-producing cells

Amount of the CBNF containing cells in relation to all measured cells

Percentage of GFP containing cells in relation to all measured cells

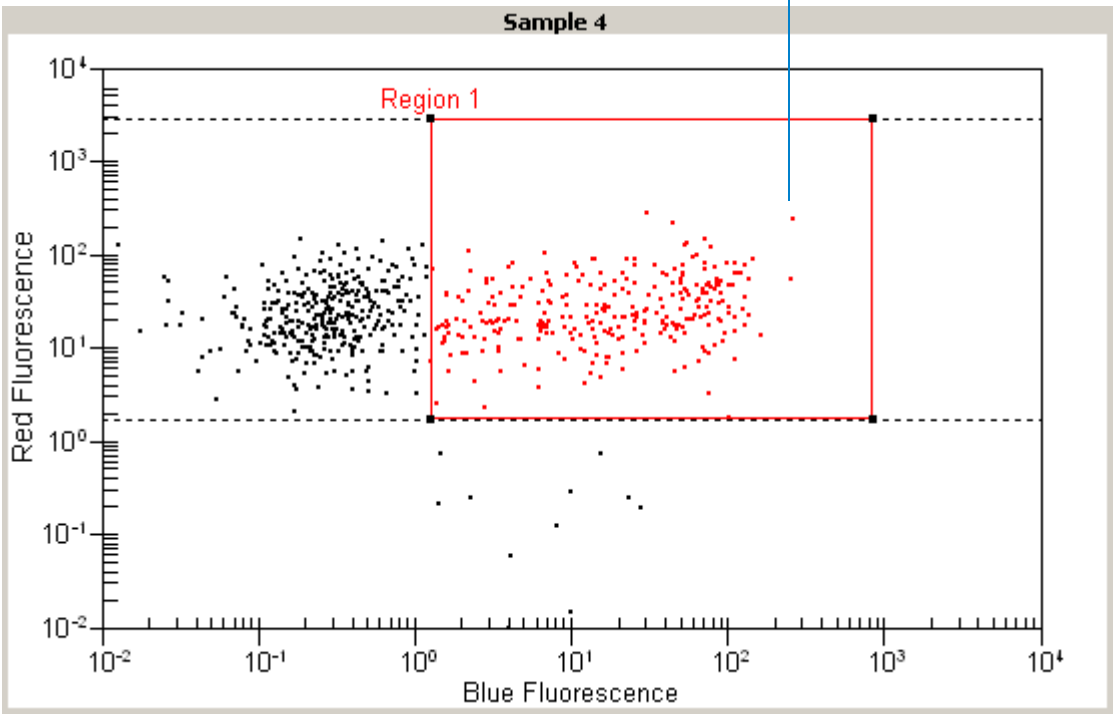
		Marker	% of gated	% total	# Events
▶	●	Gated by CBNF	-	84.7	557
	●	GFP on subset	60.9	51.5	339

Percentage of GFP containg cells in relation to the cells gated by the CBNF marker

Dot plot evaluation

If you switch to the *Dot Plot* tab, one region is displayed in the dot plot. The red fluorescence values of the region are related to the marker in the red histogram, the blue fluorescence values to the marker in the blue histogram. Corresponding to the histogram evaluation, high blue fluorescence and high red fluorescence indicate living GFP-producing cells. See the following example.

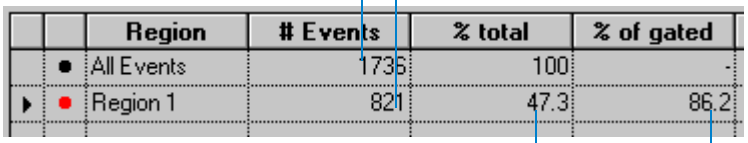
Cumulation of high blue and high red fluorescence indicates living GFP expressing cells



The results of the dot plot evaluation are displayed in the result table.

Events covered by the region

All measured events



	Region	# Events	% total	% of gated
●	All Events	1736	100	-
▶ ●	Region 1	821	47.3	86.2

Amount of cells with high CBNF fluorescence and high GFP fluorescence in relation to all measured events

Amount of cells with high GFP fluorescence in relation to the amount of CBNF-stained cells.

Working with Chip Data and Assays

You can make efficient use of the chip and assay data generated by the 2100 expert software, if you know the following fundamentals and operating techniques:

- "2100 Expert Data Overview" on page 259
- "Handling Assays" on page 262
- "Handling Chip Data" on page 267
- "Organizing, Backing up, and Archiving 2100 Expert Data" on page 269
- "Importing Data" on page 271
- "Exporting Data" on page 277
- "Printing Reports" on page 286
- "Configuring Tables" on page 293

2100 Expert Data Overview

The 2100 expert software manages data in the following different formats:

- Assay files (.xsy)
- Chip data files (.xad)
- Comparison files (.xac)
- Verification result files (.xvd)
- Diagnostics result files (. xdy)
- Result flagging rule files (.xml)

Assay files

Assay files (.xsy) contain the following information:

- Data acquisition and analysis setpoints

  Acquisition setpoints are instrument commands and acquisition parameters. Analysis setpoints are evaluation parameters, some of which you can modify.

- Assay information

  All parameters defined by the assay, such as assay type, title, and version.

- Chip and sample information

  These are chip comments, sample names and comments.

- Marker and region definitions (flow cytometric assays only)

  Included are associated parameters, such as the gating direction.

- Ladder table and peak table (electrophoretic assays only)
- Result flagging rules (electrophoretic assays only)

Chip data files

Chip data files (.xad) contain the following information:

- Measurement results

  After each chip run, the measurement results—also called "raw data"—are automatically saved in a new chip data file. Electrophoretic measurement results are pairs of migration time and fluorescence intensity values, flow cytometric measurements are triplets of migration time, red fluorescence, and blue fluorescence.

- Base assay information

  Because a chip run is always based on an assay file, *all* information from the assay file becomes part of the chip data file.

- Run log

  Events occurring during the chip run, such as the start and end time, or any errors or problems are entered in a "run log", which is also saved in the chip data file.

- Evaluation information

  These are modifications you made during data evaluation, such as modified gel coloring, manually set markers, manual integration, modified setpoints, modified result flagging rules, or definitions of new markers and regions.

Comparison files

You can compare the measurement results from different chip runs (electrophoretic chip data files of same assay class only) by collecting samples from different chip data files (.xad) and storing them in a comparison files (.xac). It is then possible to overlay electropherograms of these samples, for example, but also to compare gel-like images or data tables.

Verification result files

Verification result files (.xvd) contain results of qualification tests regarding the bioanalyzer hardware and software. The files are stored in the "..\validation" subfolder of the 2100 expert installation directory. For each verification run, an .xvd file is generated.

Date and time of the verification run are included in the file name. Example: "Verification_23-05-2005_10-28-40.xvd".

Diagnostics result files

To ensure proper functioning of the bioanalyzer hardware you should run hardware diagnostics tests on a regular basis. The results of these hardware tests are stored in diagnostics results files (.xdy) in the "..\diagnosis" subfolder of the 2100 expert installation directory.

Result flagging rule files

You can export and import result flagging rules from other assay or chip data files. Result flagging rules are stored in .xml files.

Handling Assays

Predefined Assays

Predefined assays are provided with 2100 expert. They are meant and prepared for measurements using the available LabChip kits.

Predefined assays such as *Apoptosis* or *DNA 1000* are write-protected. Although you can open predefined .xsy files and edit some of their properties, you cannot save any changes under the original file name.

Custom Assays

You can derive your own assays from the predefined assays as described in "How to Create a Custom Assay" on page 264.

The main benefit of custom assays is that you have to do the following *only once* in the assay file, instead of doing it again and again in the chip data files:

- Modify assay setpoints (data analysis setpoints).
- Enter information on chip, samples, and study.

  For example, if your sample names are to be the same for a series of chip runs.
- Define rules for result flagging (electrophoretic assays only).
- Define markers and regions for evaluation (flow cytometric assay *Generic* only).

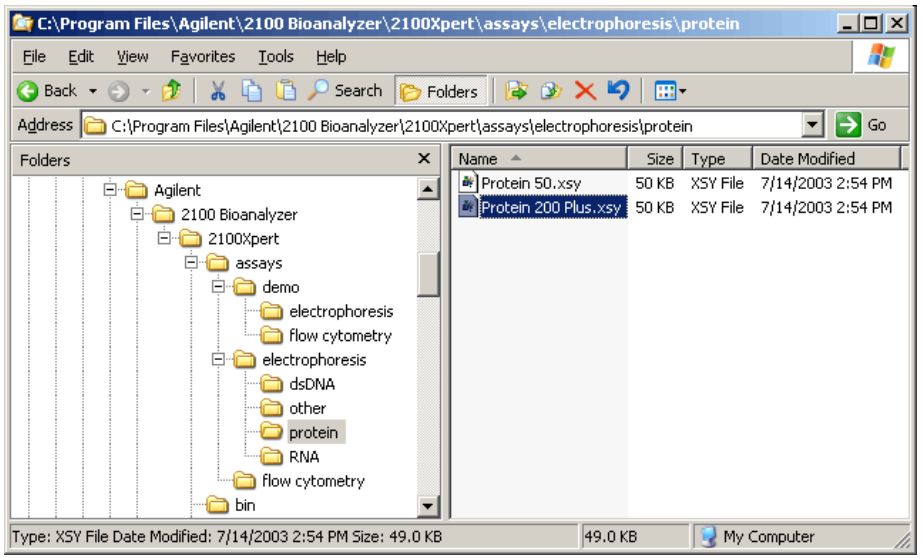
  For example, if you want to adjust marker positions and use these for future chip runs.

You can modify custom assays at any time. See "How to Modify a Custom Assay" on page 265.

TIP

If you just want to view the properties of a custom assay, you can open the assay file in read-only mode, ensuring you do not make accidental changes.

The *Assays* menu is dynamically built from the structure and contents of the "..\assays" subdirectory of the 2100 expert installation folder.



TIP

You can add items to the *Assays* menu by placing assay (.xsy) files—your own assays, for example—in subdirectories of the "..\assays" directory.

How to Create a Custom Assay

To create a custom assay:

1 Switch to the *Assay* context.

2 From the *Assays* menu, select an assay.

– OR –

Select *File* > *Open* and open an assay (.xsy) file.

The file appears in the *Tree View Panel*.

NOTE

If you want to create a new flow cytometric assay with free gating direction or with more than one marker or region, open and modify the assay "Generic.xsy".

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3 Switch to the *Assay Properties Tab* to modify the assay setpoints if required.

4 Switch to the *Chip Summary Tab* to enter chip, sample, and study information.

5 For flow cytometric assays, define markers and regions on the *Histogram Tab (Single/Grid View)* and *Dot Plot Tab (Single/Grid View)*.

6 For electrophoretic assays, define flagging rules on the *Result Flagging Tab*.

7 Select *File* > *Save As* to open the *Save As* dialog box.

8 Under *Save as type*, select *(.xsy)*, and enter a name and location for the new assay.

9 Click *Save* to create the new assay.

How to Modify a Custom Assay

NOTE

You cannot save modifications to predefined assays such as *Apoptosis* or *DNA 1000*.

To modify a custom assay:

1. From the *File* menu select *Open*.

   The *Open* dialog box appears.

2. Select an assay (.xsy) file and click *Open*.

   The assay appears in the *Tree View Panel* and the *Assay Properties Tab* is displayed.

3. Modify the assay by making changes on the following tabs:

   – Modify assay setpoints on the *Assay Properties Tab*.

   – Modify or enter additional chip, sample, and study information on the *Chip Summary Tab*.

NOTE

The study description is stored in the 2100 expert system file. Altering the study description of an assay will not affect the entries in the data files that were previously generated from this assay. To update this information in the data files, too, they must be opened, and the study must be assigned again.

   – For flow cytometric assays, define or modify markers and regions on the *Histogram Tab (Single/Grid View)* and *Dot Plot Tab (Single/Grid View)*.

- For electrophoretic assays, define or modify flagging rules on the *Result Flagging Tab*.

4 From the *File* menu select *Save* to save the method with the current name or *Save as* to save it with a new name.

Handling Chip Data

Chip data (.xad) files are automatically generated at the end of a chip run. The .xad files are given names that correspond to the choices you have made in the *Options* dialog box (see "How to Specify Data File Names and Directories" on page 300).

Modifying and saving chip data files

2100 expert allows to re-open chip data files, reanalyze them using different evaluation parameters and store the new results. You can save modifications either to the original file (*File* > *Save*) or under a new file (*File* > *Save As*).

NOTE

Raw data acquired from the bioanalyzer is *not* changed—only evaluation and display of the results can be changed and saved.

If you alter the data shown in any way after it has been saved and try to exit the program or switch to a different context (to acquire new data, for example), a dialog box will appear asking whether or not you wish to save the changes.

Opening chip data files as read-only

A chip data file can be opened as read-only; the *Title Bar* will show "(Read-Only)" at the end of the filename. The read-only file can be edited but may not be saved under the same name. If you attempt to save an edited read-only file, and error message will be displayed explaining that the file is a read-only file.

The benefit of opening chip data files as read-only is to prohibit you or other users from making changes that would alter the file in any way. Because the 2100 expert software allows you to open chip data files, modify data, and save them, you may prefer to ensure that the original parameters that were used to create the file are not altered.

Organizing, Backing up, and Archiving 2100 Expert Data

As you begin to work with the 2100 expert software, it is good practice to organize your files. If you are not the only user of the bioanalyzer, creating a directory within which to save your files is recommended; having each person save files to their own directory will speed the process of finding a particular file when someone wishes to examine the data again. Even if only one person uses the 2100 expert software, it is still wise to review your files periodically, archive files you are no longer using but wish to save, and discard unneeded files.

Organizing 2100 Expert Data

Each user in your laboratory may want to specify a particular prefix that will easily differentiate their data files from any others.

To do this, switch to the *System* context, go to the *System Wide Settings* tab, and select *Data Files* in the tree navigation. Then activate the *Prefix* check box, and edit the prefix string as you require. Note that you can also modify the file prefix before you start a chip run. Additionally, you may specify that a new directory is created each day for storage of that day's runs. To do this, activate the *Create Daily Subdirectories* check box on the same screen.

Backing up 2100 Expert Data

It is strongly recommended to save your files to a backup disk or on CD/DVD on a regular basis. This allows to retrieve the data in case of a system crash or other cases of data loss.

Archiving 2100 Expert Data

The difference between archiving and backing up is that in the archiving process the data will be removed from its original place and moved while during the backup process only a copy is taken (depending on the tools you use).

It is a good idea to periodically archive your files to a CD/DVD to remove them from your hard disk. Depending on the amount of hard disk space available to the 2100 expert software, you may need to clear space on your hard drive to ensure that you will have enough room to save upcoming chip run data.

Importing Data

When working with assay (.xsy) or chip data (.xad) files, you enter specific information that you may want to reuse. To support the reuse of data, 2100 expert has the following import capabilities:

- "Importing Bioanalyzer Files" on page 272
- "Importing Data Analysis Setpoints" on page 273
- "Importing Chip, Sample, and Study Information" on page 275

You can import result flagging rules definitions for result flagging into electrophoretic assay or chip data files:

- "Importing Result Flagging Rules" on page 276

Importing Bioanalyzer Files

You can import data, assay and method files that were generated with other Agilent 2100 bioanalyzer systems. You can even import data and assay files from the older *Bio Sizing* and *Cell Fluorescence* software applications.

To import assay files:

1. Switch to the *Assay* context.
2. From the *File* menu, select *Import* to display the *Open* dialog box.
3. Select a file of any of the following types:
   - .xsy (2100 expert assay file)
   - .asy (*Bio Sizing* assay file)
   - .csy (*Cell Fluorescence* assay file)
4. Click *Open*.

   The imported file appears in the *Tree View Panel*, and the *Assay Properties* tab shows information about the assay.

   Upon importing, the file gets converted to a new 2100 expert assay file (.xsy).

To import chip data files:

1. Switch to the *Data* context.
2. From the *File* menu select *Import* to display the *Open* dialog box.
3. Select a file of any of the following types:
   - .xad (2100 expert assay file)

– .cld (*Bio Sizing* assay file)

– .cad (*Cell Fluorescence* assay file)

4 Click *Open*.

The imported file appears in the *Tree View Panel*, and the electropherogram grid view shows an overview of all samples.

Upon importing, the file gets converted to a new 2100 expert chip data file (.xad).

Importing Data Analysis Setpoints

You can import data analysis setpoints from other assay (.xsy) or chip data (.xad) files of the same type.

Note the following when importing:

- Electrophoresis files to be imported must be of the same assay type. This means that you cannot import setpoints from a DNA 1000 assay into a DNA 500 assay, for example.
- Flow cytometry files to be imported can be of any flow cytometric assay type, but the import will change the type of the current file to *Generic*.

To import data analysis setpoints:

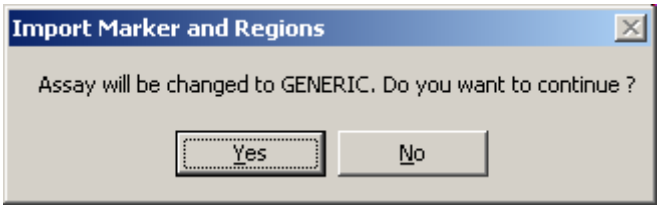
1 On the *Assay Properties* tab, click *Import Setpoints*.

2 The *Open* dialog box appears.

3 Select the file from which you want to import, and click *Open*.

NOTE

For flow cytometry files, the import will *delete* all existing markers and regions in the current file, and change the current assay to a *Generic* assay. A message box appears that prompts you to confirm this change.



4 Click *Yes*.

NOTE

Importing data analysis setpoints *overwrites* all current setpoint values.

- All files: the setpoint values are updated in the setpoint explorer, and immediately applied to the measurement results (if any).
- Flow cytometry files: the new markers and regions are now available for evaluation, and calculations based on the new markers and regions are immediately done.

5 From the *File* menu, select *Save* to make the changes permanent.

Importing Chip, Sample, and Study Information

On the *Sample Information* and *Study Information* sub-tabs of the *Chip Summary* tab, you can enter names and comments regarding chip, samples, and study. The information you enter here may be very similar for further chip runs or other assays. Once you have entered the information, you can export it into a separate file (see "Exporting Chip Run Data" on page 278), which you can then import into other chip data (.xad) or assay (.xsy) files instead of typing it anew.

The import/export files can have the extension .txt or .csv, and have a fixed form, which differs for electrophoretic and flow cytometric assays.

To import chip, sample, and study information:

1. On the *Chip Summary* tab, click *Import*.
2. The *Import Sample Information* dialog box appears.
3. Select the file that contains the information you want to import, and click *Open*.

   The Sample Information and *Study Information* sub-tabs update to show the imported data.
4. From the *File* menu, select *Save* to make the changes permanent.

Importing Result Flagging Rules

You can import result flagging rules into electrophoretic assay (.xsy) or chip data (.xad) files. Result flagging rules can be stored in .xml files (see "Exporting Result Flagging Rules" on page 285).

To import result flagging rules:

1. Open an electrophoretic assay or chip data file in the respective context.
2. Switch to the *Result Flagging Tab*.
3. In the *Result Flagging* toolbar click .

   The *Load Rules* dialog box appears.
4. Select the .xml file that contains the set of result flagging rules, and click *Open*.

   The imported rules appear in the rule list.

Exporting Data

2100 expert allows you to export the results of your chip runs in a variety of formats. The exported data can be used for further evaluation with other applications, such as text processors, graphic tools, MS Excel®, or flow cytometry applications.

You can export the chip run data of the currently loaded file either manually or automatically:

- "Exporting Chip Run Data" on page 278
- "Exporting Chip Run Data Automatically" on page 280

If you want to export only parts of your measurement results:

- "Exporting Tables" on page 281
- "Exporting Graphs" on page 282
- "Copying Graphs and Tables into the Clipboard" on page 283

Information that you have entered to document a chip run can be exported for reuse in future chip runs:

- "Exporting Chip, Sample, and Study Information" on page 284

From electrophoretic assay or chip data files, you can also export rule definitions for result flagging:

- "Exporting Result Flagging Rules" on page 285

Exporting Chip Run Data

To export chip run data:

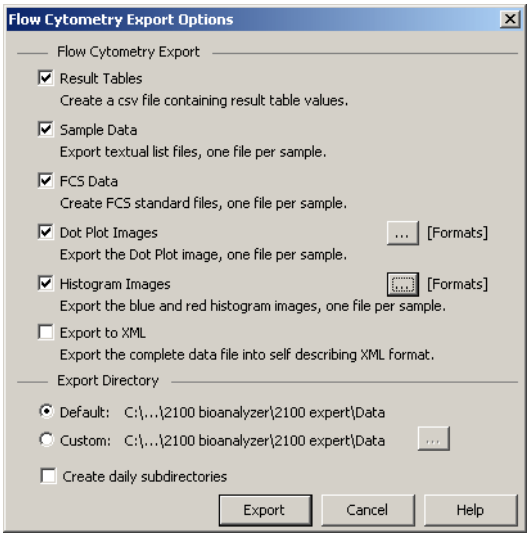
1 Switch to the *Data* context.

2 In the *Tree View Panel*, select a chip data (.xad) .file or load a file.

3 From the *File* menu, select *Export.*

If you selected an electrophoretic chip data file, the *Electrophoresis Export Options* dialog box appears.

If you selected a flow cytometric chip data file, the *Flow Cytometry Export Options* dialog box appears.


Flow Cytometry Export Options
Flow Cytometry Export
Result Tables
Create a csv file containing result table values.
Sample Data
Export textual list files, one file per sample.
FCS Data
Create FCS standard files, one file per sample.
Dot Plot Images
[Formats]
Export the Dot Plot image, one file per sample.
Histogram Images
[Formats]
Export the blue and red histogram images, one file per sample.
Export to XML
Export the complete data file into self describing XML format.
Export Directory
Default: C:\...\2100 bioanalyzer\2100 expert\Data
Custom: C:\...\2100 bioanalyzer\2100 expert\Data
Create daily subdirectories
Export
Cancel
Help

4 Select the export categories, and specify a target directory.

NOTE

Keep in mind that exporting a chip data file can require up to 20 MB of disk space. In particular, exporting electropherograms and gel-like images as .tif or .bmp files may take up a lot of disk space.

5 Click *Export*.

Several system dialog boxes appear, one for each export category, allowing you to check and modify names and locations of the export files. Clicking the *Save* button in these dialog boxes finally starts the export.

TIP

Chip run data can be automatically exported every time a chip run has finished. Refer to "Exporting Chip Run Data Automatically" on page 280 for details.

Exporting Chip Run Data Automatically

NOTE

Keep in mind that exporting a chip data file can require up to 20 MB of disk space. In particular, exporting electropherograms and gel-like images as .tif or .bmp files may take up a lot of disk space.

To enable and configure automatic export:

1. Switch to the *System* context and select *Auto Export* in the tree navigation.
2. Activate the *Auto Export* check box.
3. Specify the export categories that are to be included in the exported files for electrophoretic and flow cytometric chip runs.
4. Switch to *Default Export Directories* in the tree navigation and specify the target directories.

From now on, chip run data is automatically exported every time a chip run has finished.

NOTE

If you stop a chip run, auto export does *not* take place.

Exporting Tables

You can export:

- Result tables, peak tables, fragment tables, and ladder tables as .csv files or .xls files.
- Log book tables as .html or .txt files.

To export a result table, peak table, fragment table, or ladder table:

1. On the *Assay Properties*, *Electropherogram*, *Gel*, *Histogram*, or *Dot Plot* tab, right-click the heading row of a table.
2. From the context menu, select *Export*.

   The *Save As* dialog box appears.
3. Enter a file name and choose the destination directory.
4. Select .csv or .xls as export file format.
5. Click *Save*.

TIP

Result tables can be automatically exported every time a chip run has finished. Refer to "Exporting Chip Run Data Automatically" on page 280 for details.

To export a log book table:

1. On the *Log Book* tab, right-click a table.
2. From the context menu, select *Export*.

   The *Export Data* dialog box appears.

3 Click the ... button to specify the file name, the destination directory, and the file type. You can choose between *HTML file* for .html output and *Tabbed text file* for .txt output.

4 Specify whether you want to export the *Selected rows only* or *All visible rows*.

5 Click *OK*.

Exporting Graphs

You can export graphs as individual graphic files. This applies to all graphs that can be displayed in 2100 expert such as electropherograms or dot plots.

To export a graph:

1 Right-click the graph, and select the appropriate entry (e.g. *Save Gel* or *Save Electropherogram*) from the context menu.

– OR –

Click the button in the toolbar.

The *Save As* dialog box appears.

2 Under *File name*, enter a name and choose the destination directory.

3 Under *Save as type*, select a graphic file format: .bmp, .jpg, .wmf, .tif or .gif.

4 Click *Save*.

The graph is written to the specified file.

Note the following:

- Histograms: only one histogram graph is exported, either the red or the blue histogram.
- Electropherograms: if the grid view is active, an overview image of the electropherograms (of *all* samples and the ladder) is exported.

TIP

Electropherograms, gel-like images, histograms, and dot plots can be automatically exported every time a chip run has finished. Refer to "Exporting Chip Run Data Automatically" on page 280 for details.

Copying Graphs and Tables into the Clipboard

You can copy graphs into the clipboard. This applies to all graphs that can be displayed in 2100 expert, such as electropherograms or dot plots.

You can also copy tables (or parts of tables) into the clipboard. This applies to most of the tables that can be displayed in 2100 expert, such as result tables or log book tables.

To copy a graph or table into the clipboard:

1 Right-click the graph or table (region).

2 From the context menu, select *Copy Gel/Copy Electropherogram* (graphs) or *Copy To Clipboard* (tables).

– OR –

Click the button in the toolbar.

You can now switch to a word processing, spreadsheet, graphics, or other application, and paste the graph or table there.

Exporting Chip, Sample, and Study Information

On the *Sample Information* and *Study Information* sub-tabs of the *Chip Summary* tab, you can enter names and comments regarding chip, samples, and study. The information you enter here may be very similar for further chip runs or other assays. Once you have entered the information, you can export it into a separate file, which you can then import into other chip data (.xad) or assay (.xsy) files instead of typing it anew.

The import/export files can have the extension .txt or .csv, and have a fixed form, which differs for electrophoretic and flow cytometric assays.

To export chip, sample, and study information to a file:

1. On the *Chip Summary* tab, click *Export*.

   The *Export Sample Information* dialog box appears.

2. Specify a file name and location for the file to which you want to export.

3. Click *Save*.

Exporting Result Flagging Rules

You can export result flagging rules for reuse in other electrophoretic assay (.xsy) or chip data (.xad) files (see "Importing Result Flagging Rules" on page 276). Result flagging rules are stored in .xml files.

To export result flagging rules:

1. Open the electrophoretic assay or chip data file with the desired result flagging rules in the respective context.
2. Switch to the *Result Flagging Tab*.
3. In the *Result Flagging* toolbar click .

   The *Save Rule Definitions* dialog box appears.
4. Browse for a folder where you want to store the rules, and specify a name for the .xml file.
5. Click *Save*.

Printing Reports

For documentation and presentation purposes, you can print reports for assay (.xsy), chip data (.xad), verification results (.xvd), and comparison (.xac) files.

You can print all reports manually, see "How to Print a Chip Run Report" on page 287. When printing manually, a preview function allows you to view the printout before starting the print job.

The 2100 expert program can also be set to print customized chip run reports automatically at the end of the run. These reports can be set up to contain different information (settings for the manual and automatic print functions are maintained separately). See "How to Turn on and Configure Automatic Printing of Chip Run Reports" on page 291 for more information.

TIP

Beside sending reports to a printer, you can also create .pdf and .html files.

How to Print a Chip Run Report

The following information can be included in a chip run report:

- You can always include:
  - Run summary—general data about the assay, and sample information.
  - Assay details—complete list of data analysis setpoints.
  - Run Logbook
  - Signature Logbook
  - Audit Trail
- For *flow cytometric* chip data files (.xad) you can include:
  - Dot plot summary—all regions shown in an overview.
  - Dot plot statistics—all statistical data of the result table.
  - Histogram summary—all histograms shown in an overview.
  - Histogram statistics—all statistical data of the blue and red histograms' result tables.
- For *electrophoretic* chip data files (.xad), depending on the assay type you can include:
  - Electropherograms
  - Gel-like image
  - Result tables
  - Standard curve
  - Calibration curve

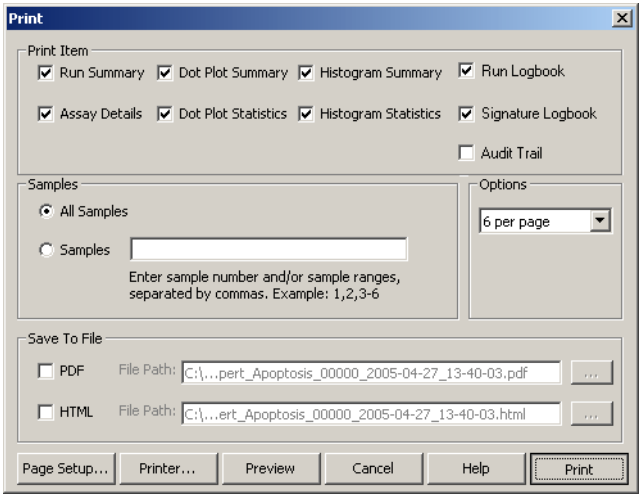
To print a report:

1 Switch to the *Data* context.

2 In the *Tree View Panel* select the chip data (.xad) file you want to generate a report of.

3 From the *File* menu select *Print*.

Depending on the file type different dialog boxes appear.



4 You generally have the following possibilities:

- select the items to be included in the report from the *Print Item* section
- select the wells to be included from the *Wells* section
- select the appropriate options
- specify whether you want to generate the report as a file (PDF or HTML)

NOTE

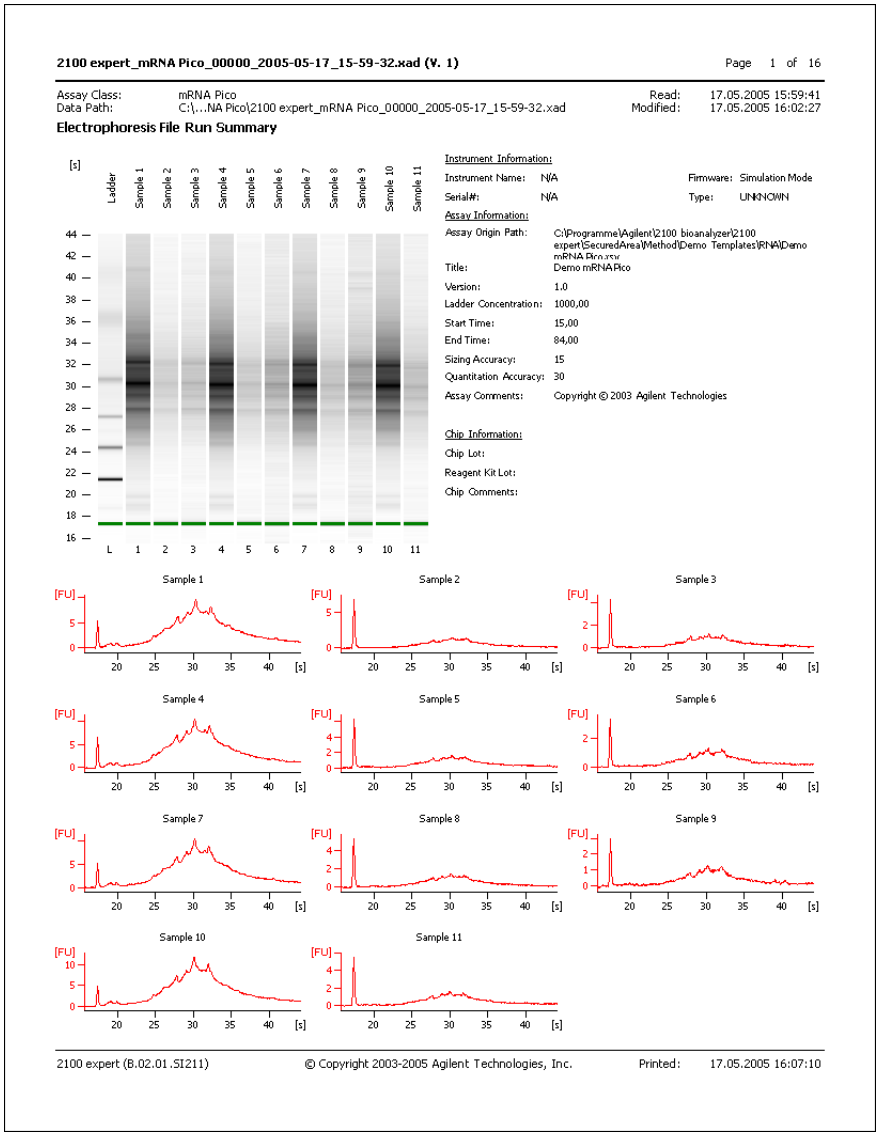
Your selections here are separate from the *Auto Print* selections (they do not affect each other). Both are used by default the next time you print (even after restarting the program).

5 Use the *Page Setup* and *Printer* buttons to access system dialog boxes, allowing you to select a printer, and specify the print medium and page layout.

6 Click the *Preview* button to get a preview of the printouts or files to be generated.

7 Click *Print* to print out the pages or generate the file(s).

The following example shows the “Run Summary” part of an RNA chip run report.



2100 expert_mRNA Pico_00000_2005-05-17_15-59-32.xad (V. 1) Page 1 of 16

Assay Class: mRNA Pico
Data Path: C:\...NA Pico\2100 expert_mRNA Pico_00000_2005-05-17_15-59-32.xad
Read: 17.05.2005 15:59:41
Modified: 17.05.2005 16:02:27

Electrophoresis File Run Summary

Instrument Information:
Instrument Name: N/A
Serial#: N/A
Firmware: Simulation Mode
Type: UNKNOWN

Assay Information:
Assay Origin Path: C:\Programme\Agilent\2100 bioanalyzer\2100 expert\SecuredArea\Method\Demo Templates\RNA\Demo mRNA Pico.xsy
Title: Demo mRNA Pico
Version: 1.0
Ladder Concentration: 1000,00
Start Time: 15,00
End Time: 84,00
Sizing Accuracy: 15
Quantitation Accuracy: 30
Assay Comments: Copyright © 2003 Agilent Technologies

Chip Information:
Chip Lot:
Reagent Kit Lot:
Chip Comments:

2100 expert (B.02.01.SI211) © Copyright 2003-2005 Agilent Technologies, Inc. Printed: 17.05.2005 16:07:10

How to Turn on and Configure Automatic Printing of Chip Run Reports

A report can be automatically printed on a printer or generated as a file at the end of each chip run. Saving reports as files can be helpful for documentation purposes.

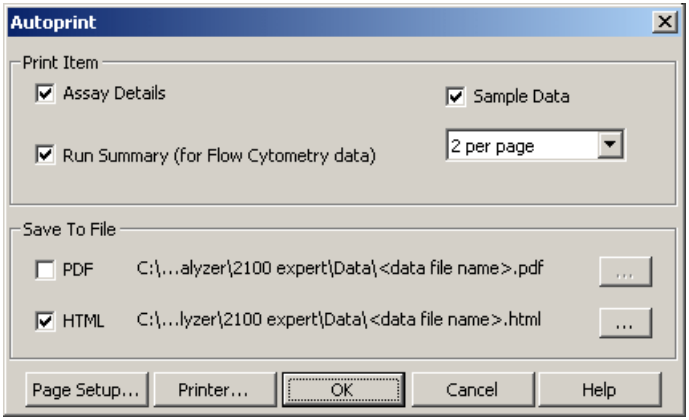
To enable and configure automatic printing:

1 Switch to the *System* context.

2 Select *Run and Result* in the tree navigation.

3 Activate the *Auto Print* check box and click the *Settings* button next to this check box.

The *Auto Print* dialog box appears.



NOTE

The *Auto Print* settings are independent from those made via the *Print* command of the *File* menu .

4 Adjust the settings:

- In the *Print Item* section, select the options that are to be included in the report.
- In the *Save To File* section, you can redirect the automatic printouts to .pdf and .html files.

  Note that no print output is generated if you select the *PDF* and/or *HTML* option.
- Using the *Page Setup* and *Printer* buttons, you can access system dialog boxes, allowing you to select a printer for the automatic print, and specify the print medium and page layout.

5 Click *OK* to confirm the automatic print settings.

Configuring Tables

2100 expert uses various tables to present data:

- Result tables
- Peak tables
- Fragment tables
- Log book tables

In some cases, you might want to reorganize the way the data is presented. To do so, you can hide or show columns, change the column sequence, and adapt the table height.

	Area	FragmentSize	Concentration	Molarity	Observations	
1	50.29	15.00	4.20	424.24	CalculatedLowerMarker	
2	29.49	472.30	0.76	2.45		
3	13.40	500.66	0.34	1.03		
4	84.13	1500.00	2.10	2.12	CalculatedUpperMarker	

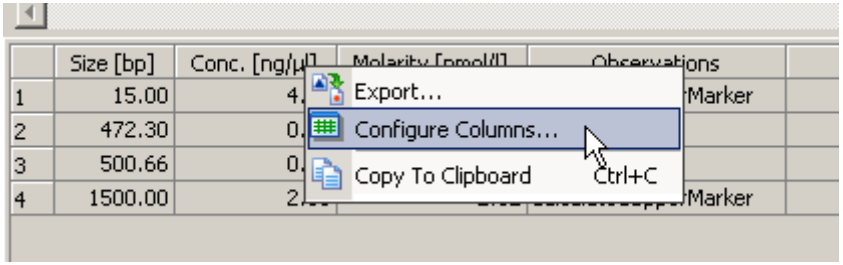
The following example demonstrates how to add the migration time to the *Peak Table*.

Showing and Hiding Columns

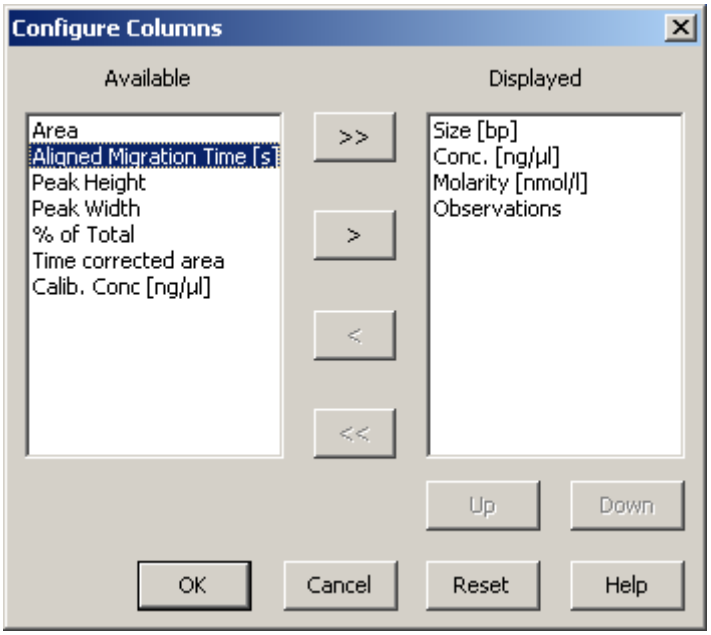
To add the *Aligned Migration Time* column to the table:

1 Right-click the heading row of a table and select *Configure Columns* from the context menu.

The *Configure Columns* dialog box opens.



2 Move any desired column headers from the *Available* list to the *Displayed* list.



3 Configure the order of the column headers in the *Displayed* list by using the *Up* and *Down* buttons.

4 Click *OK*.

A new column *Aligned Migration Time* is inserted in the table:

		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations	Aligned Migration Time [s]
1	◀	15	4.20	424.2	Lower Marker	41.00
2		22	1.55	105.6		42.72
3		55	1.23	33.8		47.44
4		104	3.90	56.7		53.04

TIP

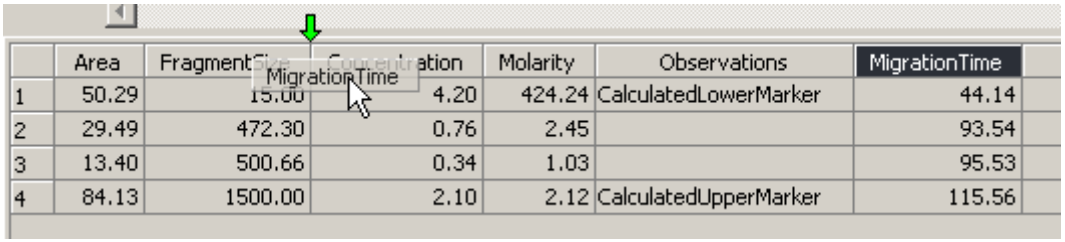
You can set the column sequence also using the *Up* and *Down* buttons in the *Configure Columns* dialog box.

To change the column sequence of a table:

1 Position the mouse pointer on a column header.

2 Click and hold the left mouse button, and drag the header cell to the desired position.

While dragging, a green arrow indicates the target position.



	Area	FragmentSize	Concentration	Molarity	Observations	MigrationTime
1	50.29	15.00	4.20	424.24	CalculatedLowerMarker	44.14
2	29.49	472.30	0.76	2.45		93.54
3	13.40	500.66	0.34	1.03		95.53
4	84.13	1500.00	2.10	2.12	CalculatedUpperMarker	115.56

3 Release the mouse button.

The column has moved to its new position:

	Area	FragmentSize	MigrationTime	Concentration	Molarity	Observations
1	50.29	15.00	44.14	4.20	424.24	CalculatedLowerMarker
2	29.49	472.30	93.54	0.76	2.45	
3	13.40	500.66	95.53	0.34	1.03	
4	84.13	1500.00	115.56	2.10	2.12	CalculatedUpperMarker

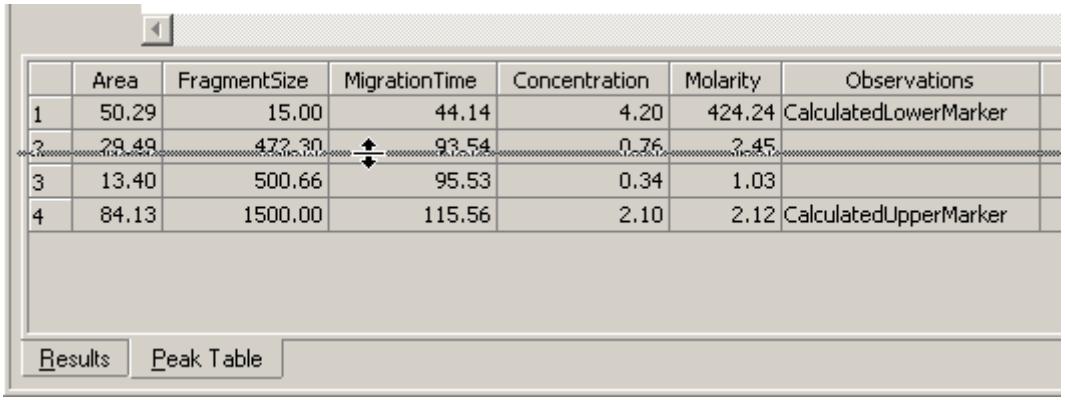
Changing the Table Height

You can customize the view by changing the height of the table.

To increase or reduce the table height:

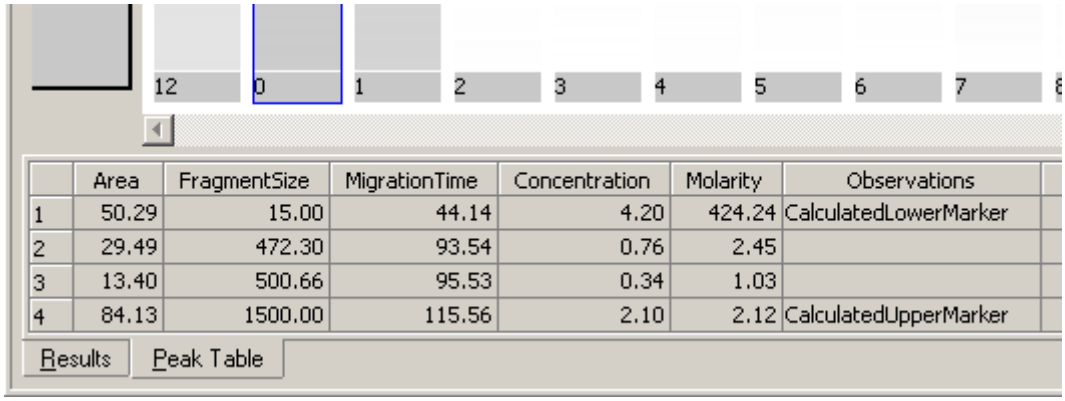
1 Position the mouse pointer above the heading row of the table and move it upwards until the cursor's shape changes to a double arrow.

2 Click and hold the left mouse button and drag up or down.



3 Release the mouse button.

In this example, the *Peak Table* freed screen space for the gel-like image above the table:



Administering System Functions

The 2100 expert software provides the following configuration options and system functions:

- Default data file names and directories can be specified. Also, settings such as for automatic printing or automatic data export can be set up. See "Configuring 2100 expert" on page 300 for details.
- Log books are provided that record all important actions and messages in the 2100 expert software. See "Using Log Books" on page 310 for details.

Configuring 2100 expert

The available options for configuring the 2100 expert software can be found in the *System* context on the *System Wide Settings* tab.

How to Specify Data File Names and Directories

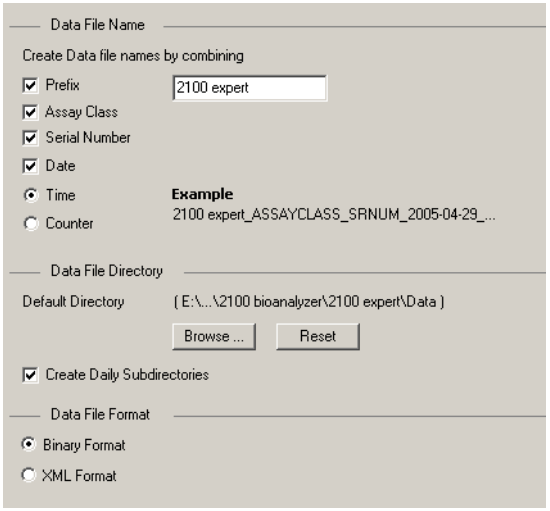
The measurement results are stored automatically when the chip run is complete. To make it easier for you to identify the chip data files, you can configure an automatic naming scheme for the files.

To specify the names and destination for generated chip data files:

1 Switch to the *System* context and select the *System Wide Settings* tab.

2 Select *Data Files* in the tree navigation.

The *Data Files* screen becomes visible:



3 In the *Data File Name* section, select the check boxes of the strings you want to insert in the file names:

Option	Meaning
Prefix	Inserts an arbitrary string to identify the data file. This string can be modified. The default file prefix is “2100 expert”.
Assay Class	Inserts the assay class in the file name. Examples: “DNA1000”, “GFP”, “Apoptosis”.

Option	Meaning
Serial Number	Inserts the serial number of the Agilent 2100 bioanalyzer instrument used for the chip run.
Date	Inserts the date of the chip run.
Time/Counter	Inserts the time of the chip run/inserts an auto-incremented 3-digit number.

4 In the *Data File Directory* section, specify the *Default Directory* where the chip data files are to be stored. Use the *Browse* button to select a directory or click *Reset* if you want to use the "..\Data" directory under the 2100 expert installation directory.

5 Optionally, you can select the check box *Create Daily Subdirectories* if you want daily subdirectories to be created.

This option helps you to better organize your chip data files. If selected, a subdirectory is created for every day in which a chip run was started. The name of the subdirectory has the format "YYYY-MM-DD", for example, "2005-01-22". All chip data files generated on this day will be stored in this subdirectory.

6 In the *Data File Format* section, select whether you want to save the data files in *Binary Format* or in *XML Format*.

7 Use the *Prefix* field to specify a default prefix for the created files. This default prefix can be changed by every analyst in the *Instrument* context for each chip run.

How to Set Run and Result Options

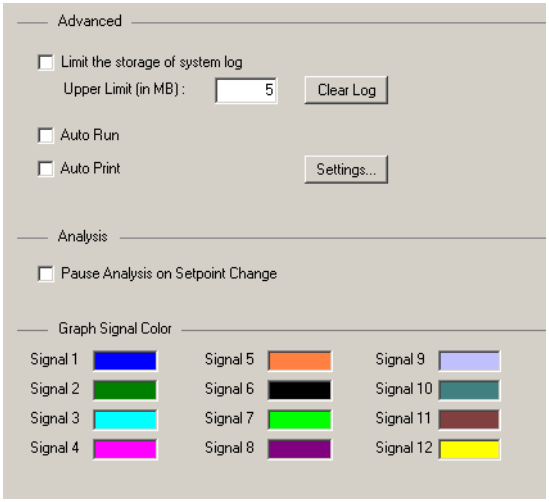
You can select several options such as to pause the analysis on setpoint changes, the maximum log file size, or the graph colors.

To set the *Run and Result* options:

1 Switch to the *System* context and select the *System Wide Settings* tab.

2 Select *Run and Result* in the tree navigation.

The *Run and Result* screen becomes visible:



3 In the *Advanced* section, you can

– select *Limit the storage of system log* if you want to limit the disk space for the system log file "SystemLogBook.log" (located in the "..\log" subdirectory), and enter an upper limit in MB.

If the limit is exceeded, a message appears that prompts you to delete or move the log file to get free disk space.

– select *Auto Run* to activate the automatic start of a chip run once the lid of the Agilent 2100 bioanalyzer is closed and a chip suiting the selected assay is detected.

– select *Auto Print* to enable the automatic report printing function.

  You can now click *Settings* to display the *Autoprint* dialog box, where you set the options for automatic printing after a chip run is complete.

NOTE

The *Auto Print* settings are independent from those made via the *Print* command of the *File* menu.

---

4 In the *Analysis* section, you can activate the *Pause Analysis on Setpoint Change* function.

  If this function is not active, the measurement results are recalculated every time after you change a setpoint. If you need to change several setpoints at once, activating this function saves you time, because the results are only recalculated when leaving the setpoint explorer or when starting the analysis manually with the start button.

5 In the *Graph Signal Color* section, click the colored rectangles to the right of the signals.

  You can now choose a new color for the selected signal in the *Color* dialog box.

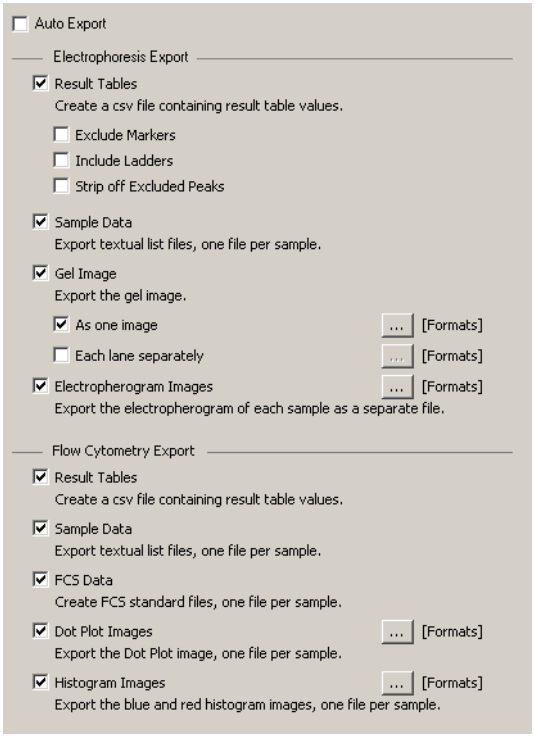
How to Set Auto Export Options

To define auto export options:

1 Switch to the *System* context and select the *System Wide Settings* tab.

2 Select *Auto Export* in the tree navigation.

The *Auto Export* screen becomes visible:



3 Activate the *Auto Export* check box, if you want a data file to be exported automatically after every chip run.

4 In the *Electrophoresis Export* section, specify which elements are to be included in the exported file for electrophoresis measurements.

5 In the *Flow Cytometry Export* section, specify which elements are to be included in the exported file for flow cytometry measurements.

6 Select *Default Export Directories* in the tree navigation and define the default directories for the various file types. Optionally, you can activate the *Create daily subdirectories* check box to automatically export the files of each day to separate directories.

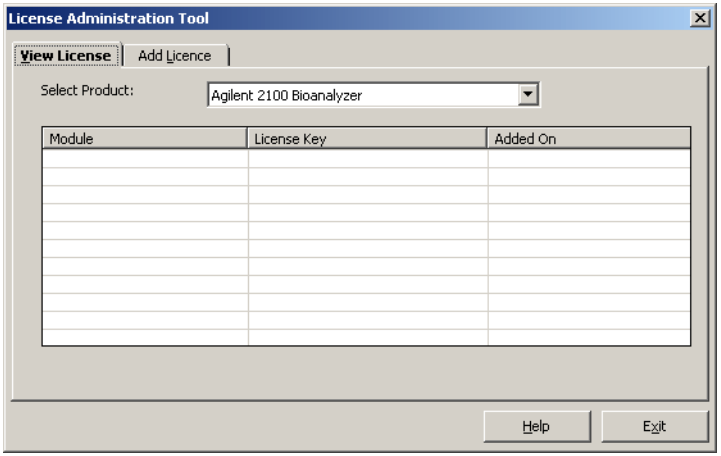
How to Activate Software Licenses

By installing the 2100 expert software you have also installed a license administration tool. This tool is used to activate the different software modules. The following licenses can be ordered separately:

- 2100 electrophoresis license
- 2100 flow cytometry license
- 2100 security pack license
- 2100 instrument control license

To activate an additional software license:

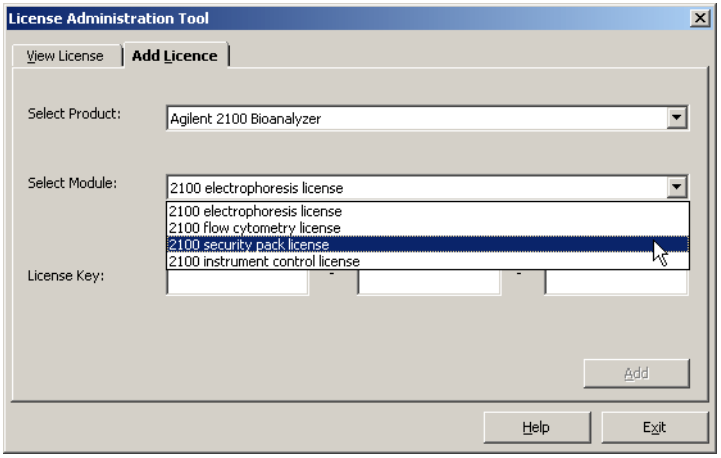
1 Select *Registration* from the *Help* menu to open the *License Administration Tool* window.



2 Switch to the *Add License* tab.

3 In the *Select Product* field, the *Agilent 2100 Bioanalyzer* must be selected.

4 In the *Select Module* field, select the license for the software module that you want to activate.



5 Enter the correct *License Key* and click the *Add* button.

A message box informs you whether the license key was added successfully.

6 If you want to add more licenses, repeat the previous two steps for every license key.

7 Click the *Exit* button to close the *License Administration Tool* window.

The licensed software modules are now activated and can be used.

NOTE

If you added the license key to activate the security pack, the 2100 expert software closes and the secured file area will be set up. Follow the instructions displayed in the different pages of the setup wizard.

NOTE

Store your license keys in a secure place and make sure you do not lose them.

Using Log Books

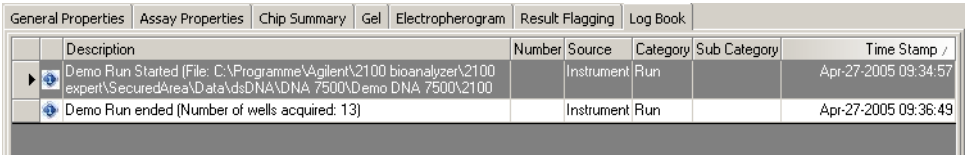
2100 expert provides several log books to document all relevant actions and changes. Due to requirements of data integrity and data security, none of the log books can be cleared.

Run Logs

The run log books can be found in the following contexts as sub-tabs of the *Log Book* tab:

- *Data* context
- *Verification* context
- *Comparison* context
- *Assay* context

They contain events such as the start and end time of a chip run, and any errors or problems that occurred during the run.

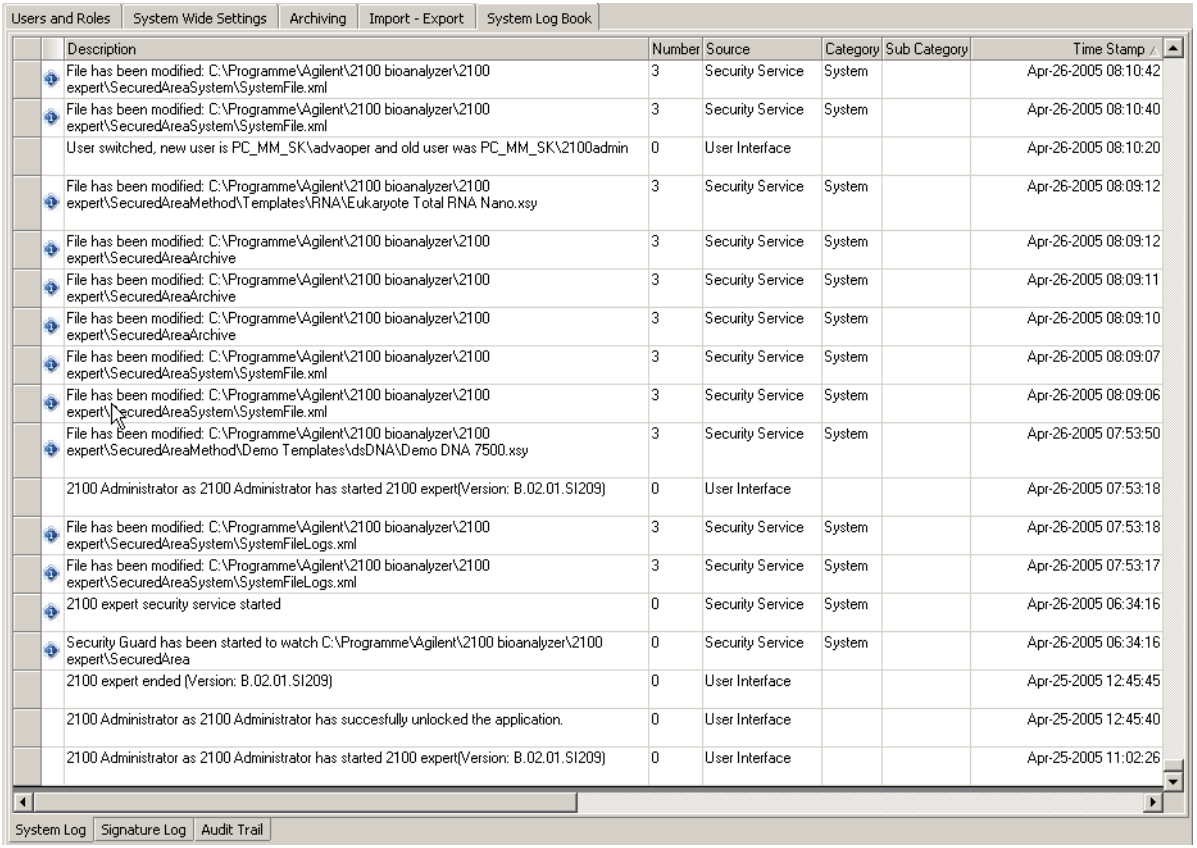


All run logs are saved in the data files within the respective context.

System Log

The system log book can be found in the *System* context as a sub-tab of the *Log Book* tab.

It includes start-up and shut-down events of the 2100 expert software, and, for example, errors or problems with the connected bioanalyzers.



The system log book is saved in `config/SystemFile.xml`. The log book entries can be exported from this file.

How to Change the Display of the Log Books

To sort a log book table:

1. Click the column header you want to sort the table by.

   The log book table is sorted by the entries in the selected column in ascending order.

2. Click the column header again to reverse the order.

To filter a log book table:

1. In the *Log Book* toolbar, click *Filter* .

   The *Filter* dialog box appears.

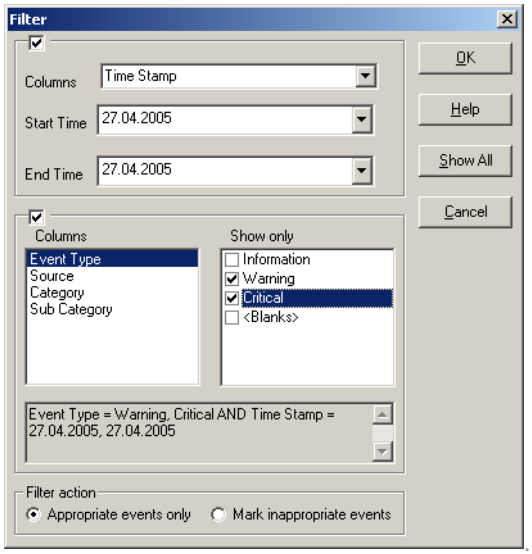
2. To define a filter for events from a specified period of time, specify a *Start Time* and an *End Time*.

3. To define a filter for events with certain entries in a column, specify the column name and the value to search for.

4. Use the *Filter Action* radio buttons to define whether only events that match the filter criteria are displayed (*FilterActionHide*) or whether those events are highlighted while the others are still listed (*FilterActionHide*).

5. Click *OK* to apply the filter to the log book table.

The filter definition in the following example excludes all events from the *Run Log* in the *Data* context with an *Event Type* other than *Critical*.



To remove the filter from a log book table:

1 In the *Log Book* toolbar, click *Reset* .

TIP

You can hide/show any of the log table columns, and re-sort the columns by right-clicking the table and selecting *Columns* from the context menu.

How to Search the Log Book

You can search the various log books for any string.

To search the *Log Book*:

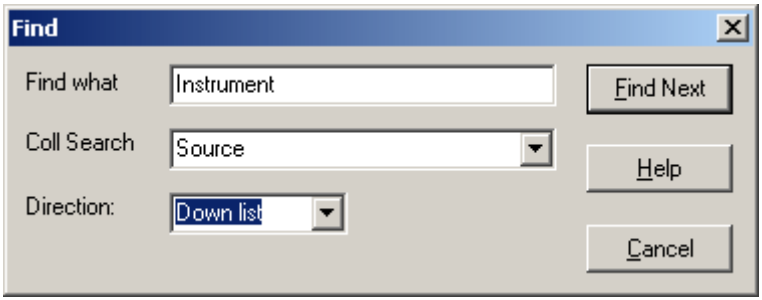
1 In the *Log Book* toolbar, click *Find* .

The *Find* dialog box appears.

2 Enter a search string in the *Find What* field.

3 Use the *Column* selection list to specify whether you want to search all columns or a particular column only.

4 Select the search *Direction*.



5 Click *Find Next*.

If the search string was found in an event, the event row gets highlighted in yellow.

NOTE

The search is *case-sensitive*!

---

6 To continue the search, click *Find Next*.

Running Instrument Diagnostics

2100 expert provides several tests to check proper functioning of the bioanalyzer hardware. You should perform the tests on a regular basis, or if incorrect measurements occur.

You can test the following:

- Generic bioanalyzer tests, which can be run with both types of cartridges (electrode or pressure cartridge)
- Bioanalyzer in combination with electrode cartridge (electrophoresis setup)
- Bioanalyzer in combination with pressure cartridge (flow cytometry setup)

Generic Bioanalyzer Tests

Diagnostics Test	Purpose
Electronics Test	Verifies proper functioning of all electronic boards in the bioanalyzer.
Fan Test	Checks if the fan is running at the appropriate speed.
Lid Sensor Test	Verifies proper operation of the lid sensor, ensuring that the laser and LED are off when the lid is open.
Stepper Motor Test	Checks for proper movement of the stepper motor.
Temperature Test	Checks if the temperature ramp-up speed of the heater plate is within specifications.

Electrode Cartridge Tests

Diagnostics Test	Purpose
HV Stability and Accuracy Test	Tests high voltage accuracy and stability of all 16 high voltage power supplies and the high voltage controller. Unused chip (DNA, RNA, or protein) required.
HV Accuracy Test (On-Load)	Check of channel-reference diode in transmission direction.
Short Circuit Test	Checks for instrument leak currents using an empty chip. Note: the limits of this test specify an ambient temperature of 25 °C and relative humidity less than or equal to 60 %. Higher temperatures or relative humidity could result in a leak current.
Electrode/Diode Test	Checks the photo diode and current-versus-voltage performance of the bioanalyzer. Electrode/diode test chip required.
Optics Test	Checks for proper alignment of internal optics and proper function of the laser and LED.
Electrophoresis Autofocus Test	Checks the focusing capability of the optical system. Autofocus test chip required.
Laser Stability Test	Measurement of stability of red laser signal.

Pressure Cartridge Tests

Diagnostics Test	Purpose
Pressure Offset Test	The vacuum system of the pressure cartridge consists of a pump and the corresponding tubes. This test calibrates the pressure sensors to zero.
Pressure Control Test	Checks if the bioanalyzer is able to hold the working pressure of -140 mbar. During the test pumps stay on, while the system tries to regulate pressure to be kept at -140 mbar. Cell Autofocus test chip required.
System Leak Test	Checks if the bioanalyzer is able to maintain a vacuum. Produces a test pressure of -100 mbar and monitors for changes. Cell Autofocus test chip required.
Flow Cytometry Autofocus Test	Checks that the optical system of the bioanalyzer is correctly calibrated. Cell Autofocus test chip required.

NOTE

With bioanalyzer model G2938A only diagnostic tests in combination with the electrode cartridge can be performed.

Test Chips

Depending on your bioanalyzer setup (electrophoresis or flow cytometry), different test chips are required to run some of the diagnostics tests. Test chip kits are part of the bioanalyzer electrophoresis set (G2947CA) and flow cytometry set (G2948CA):

Test Chip Kit for Electrophoresis Assays (reorder no. G2938-68100)

Test Chip	Comment	Quantity
Autofocus Test Chip	Values for fluorescence and offset are printed on the chip. Can be used multiple times.	1
Electrode/Diode Test Chip	Can be used multiple times.	1

Test Chip Kit for Flow Cytometry Assays (reorder no. G2938-68200)

Test Chip	Comment	Quantity
Cell Autofocus Test Chip	Required for Pressure Control Test, System Leakage Test, and Optical Drive Test. Can be used multiple times.	1

How to Run Instrument Diagnostics Tests

NOTE

Diagnostics tests cannot be run while the 2100 expert software is performing a chip run.

To run diagnostics tests:

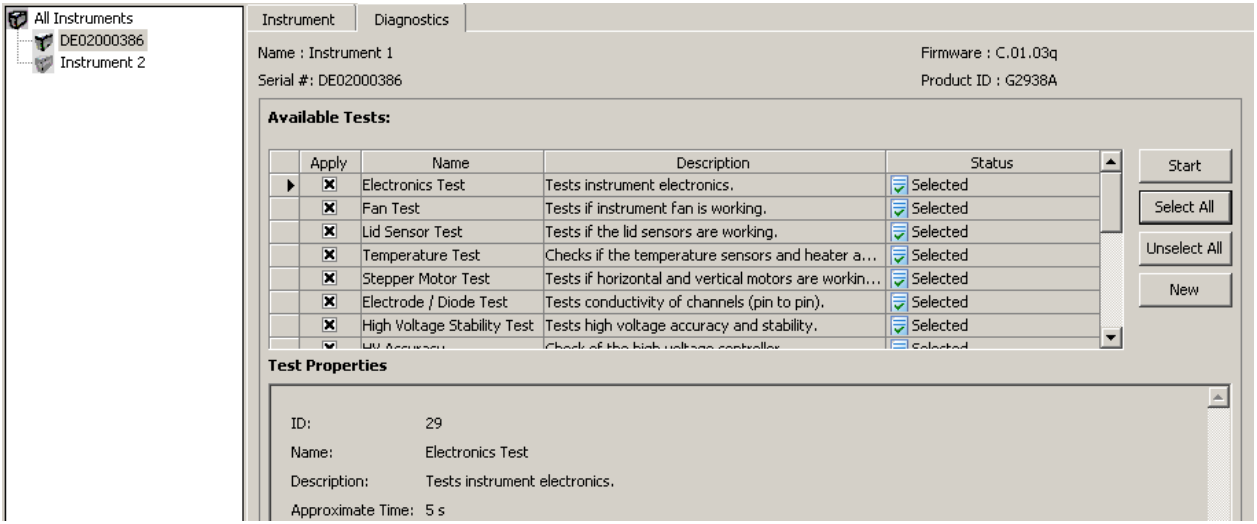
1. Switch to the *Instrument* context.
2. In the *Tree View Panel*, select the bioanalyzer on which you want to run the tests.
3. Switch to the *Diagnostics* tab.

   All available tests are displayed in the *Available Tests* list. The tests that can be executed depend on the type of cartridge that is installed in the bioanalyzer.

   The 2100 expert software will generate an error message if a wrong cartridge type is detected for the selected assay. To run the selected test please insert the requested cartridge type (see "Loading the Electrophoresis Chip into the Bioanalyzer" on page 58 or "Loading the Cell Chip into the Bioanalyzer" on page 189, respectively).

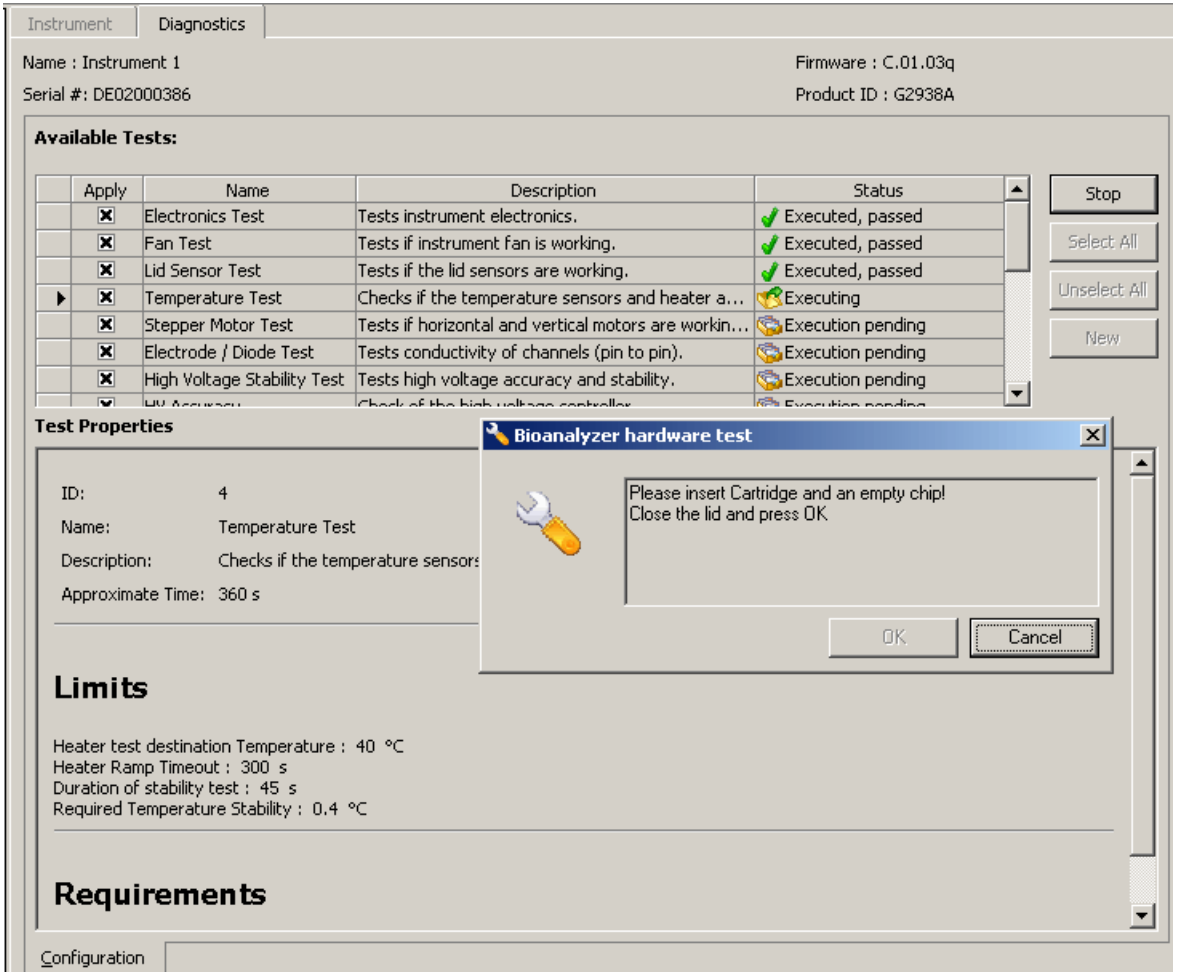
4 Select the tests you want to run:

- Select the *Apply* check boxes to select single tests.
- Click *Select All* to select all available tests.
- Click *Unselect All* to deselect all tests.



5 Click *Start*.

6 Follow the instructions given by the 2100 expert software. For example, exchange the cartridge, or put a test chip in the receptacle of the bioanalyzer when requested by the software.



All selected tests are performed.

The *Status* column indicates the status of each test:

- *Executing*
- *Execution pending*
- *Executed, passed*
- *Executed, failed*

7 If any test failed, redo the test.

8 If failures still persist, contact Agilent service.

The results of diagnostics tests are stored in .xdy files in the 2100 expert installation folder under "..\diagnosis". If tests fail, send the .xdy files to the Agilent service.

Performing Verifications

To ensure a validated Agilent 2100 bioanalyzer system, verification steps have to be performed at installation and operation level.

2100 expert allows for detailed *installation verification* and *system verification* on both the bioanalyzer hardware and software. Each verification comprises a series of tests and measurements that you can run and document in the *Verification* context of the 2100 expert software.

Installation Verification

Installation verification includes tests to verify that the bioanalyzer software and hardware are installed properly and that all electrical and pressure connections are correct.

Installation verification must be performed once after installation.

System Verification

System verification proves that the bioanalyzer system is suitable for its intended use, that is, that it will function according to its operational specifications in the selected environment.

System verification should be performed:

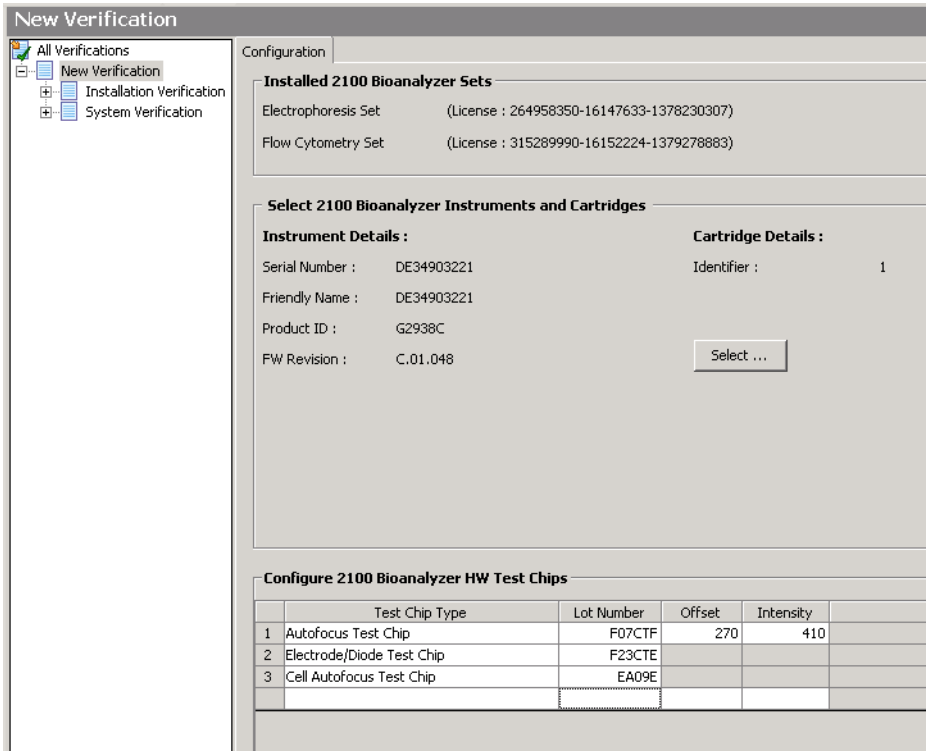
- at first use of the instrument,
- after relocating the instrument,
- after changing essential parts of the system, for example software updates or exchange of cartridges,
- after instrument repair,
- on regular time intervals.

Verification Procedure

To perform verification tests:

1. Switch to the *Verification* context.
2. From the *File* menu select *New*.
3. A *New Verification* item appears in the *Tree View Panel*.
4. Under *Cartridge Details*, click *Select* and specify details on the cartridge that is currently installed in the bioanalyzer.

5 Under *Configure 2100 Bioanalyzer HW Test Chips*, enter the test chips you will use for this verification:

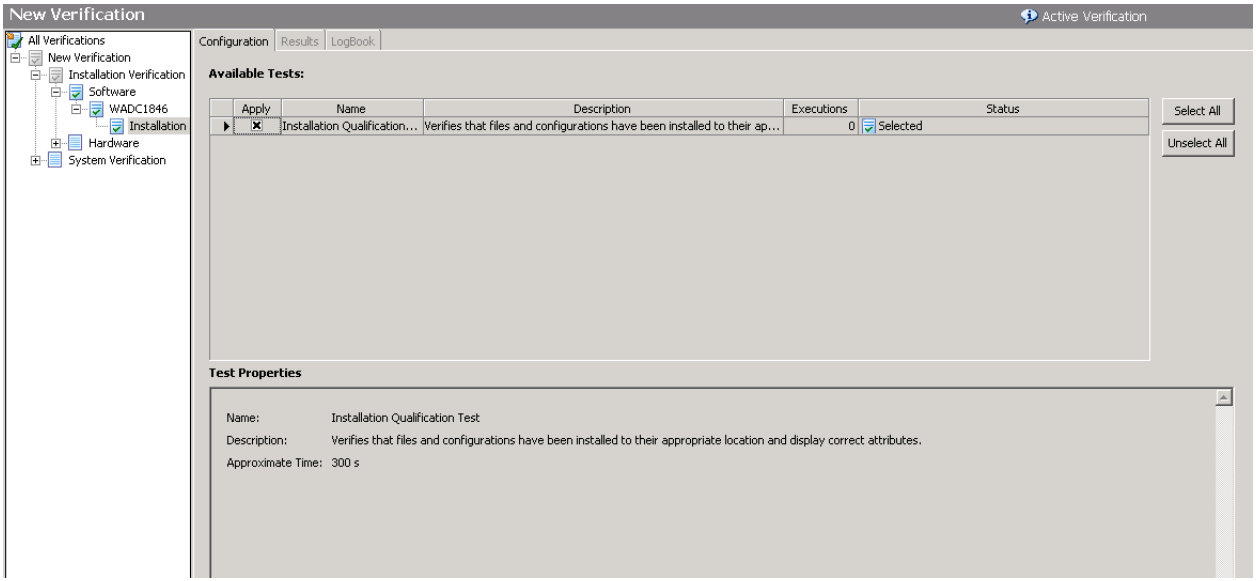


6 In the *Tree View Panel*, navigate to the test category you want to execute. Select the category via *Installation/System Verification – Software/Hardware – PC name/Bioanalyzer name – Test Category*.

NOTE

To execute hardware tests (*HW* branch) the bioanalyzer must be properly connected and switched on.

The *Configuration* tab now lets you select verification tests to be executed in the verification run:



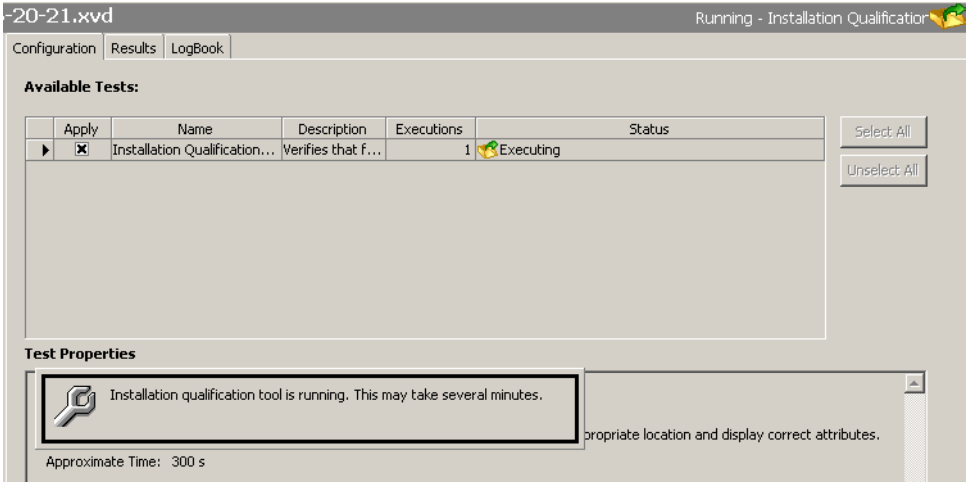
To select tests, check the *Apply* check box next to the test(s).

7 To start the selected tests, click *Start* button in the toolbar.

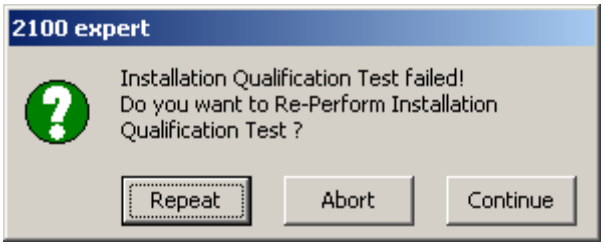
The *Save As* dialog box appears.

8 Specify a name and location for the verification results file (.xvd) and click *Save*.

The selected tests are executed.



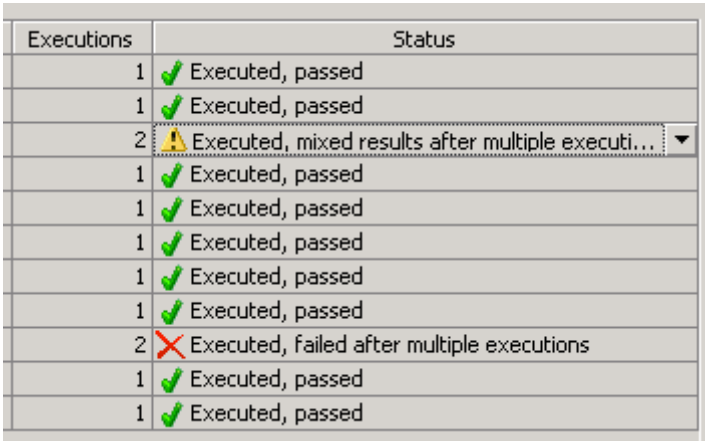
9 If a test fails, you can *Repeat* test execution, *Abort* the verification run, or skip the current test and *Continue* with the next test:



10 After all tests have been executed the following message appears:

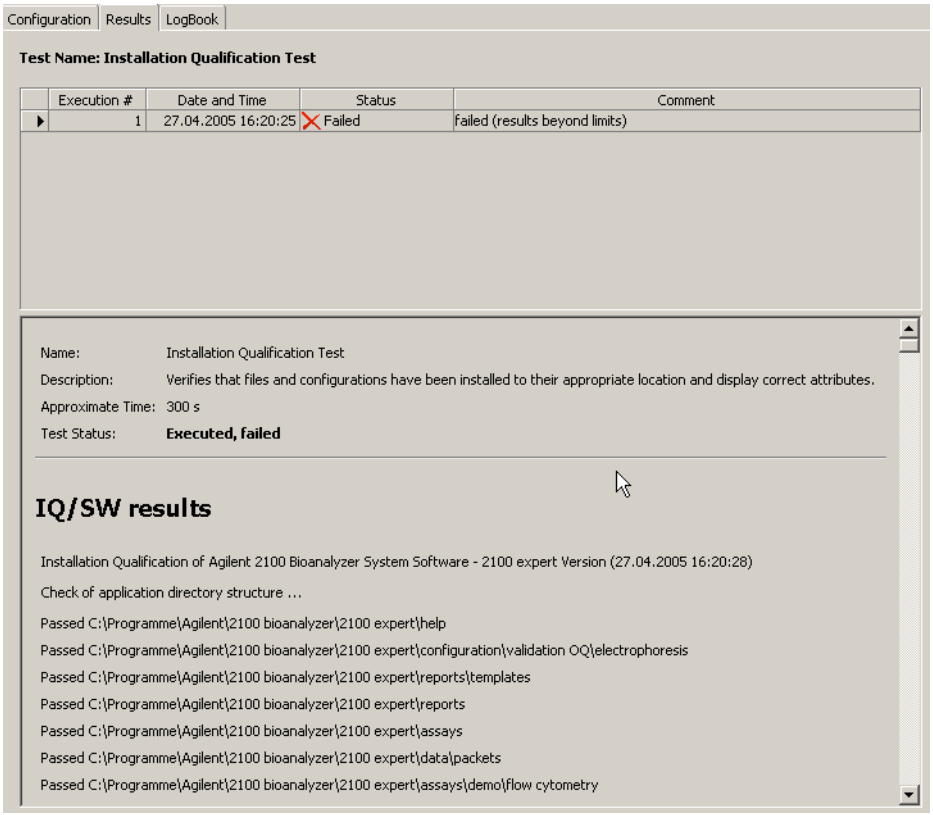
11 Click *OK*.

12 The *Status* column shows which of the tests have been run successfully, which have failed, and which have mixed results with multiple executions.



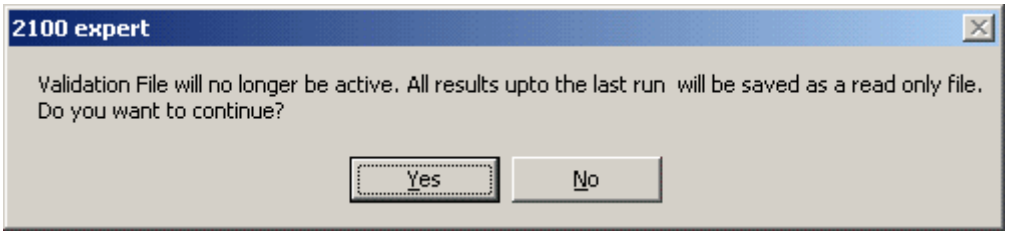
Executions	Status
1	Executed, passed
1	Executed, passed
2	Executed, mixed results after multiple executi...
1	Executed, passed
1	Executed, passed
1	Executed, passed
1	Executed, passed
1	Executed, passed
2	Executed, failed after multiple executions
1	Executed, passed
1	Executed, passed

13 To view details on test execution, select the *Results* tab.



14 You can now navigate to other test categories and execute additional verification tests.

15 When you close the verification result file (*File* > *Close*), try to switch to another context, or exit 2100 expert, the following message appears:


2100 expert
Validation File will no longer be active. All results upto the last run will be saved as a read only file.
Do you want to continue?
Yes
No

If you select *No*, you return to the *Verification* context and can run further verification tests.

If you select *Yes*, the verification result file (.xvd) is closed and becomes read-only.

NOTE

You can re-open verification result files only for viewing and printing.

TIP

Select *File* > *Print* to generate a printed report of the verification run.

Products, Spare Parts, and Accessories

To buy the following products, spare parts and accessories for the Agilent 2100 bioanalyzer, please refer to the Agilent Online Store:

http://www.agilent.com/home/buyonline.html

Bundles

- G2940CA – Agilent 2100 bioanalyzer desktop system

  Includes Agilent 2100 bioanalyzer, HP Compaq desktop PC, color printer, system software, vortexer, and accessories. Cartridge and license must be purchased separately.

- G2943CA – Agilent 2100 bioanalyzer laptop system

  Includes Agilent 2100 bioanalyzer, HP Compaq laptop PC, color printer, system software, vortexer, and accessories. Cartridge and license must be purchased separately.

Hardware

- G2938C – Agilent 2100 bioanalyzer

  Includes 1 chip priming station, 1 test chip kit, serial cable, *Installation and Safety Manual*. Cartridge and license must be purchased separately.

- G2947CA – Agilent 2100 bioanalyzer electrophoresis set

  Includes test chip kit, electrode cartridge, license key for electrophoresis assays, and start-up service.

- G2948CA – Agilent 2100 bioanalyzer flow cytometry set

  Includes checkout kit, test chip kit, pressure cartridge, license key for flow cytometry assays, and start-up service.

Software and Services

- G2946CA – Agilent 2100 expert software upgrade

  Software package for upgrade to the latest 2100 bioanalyzer system software revision.

- G2949CA – Agilent 2100 expert security pack

Additional services for Installation Qualification (IQ) and Operation Qualification/Performance Verification (OQ/PV) as well as assay consulting are available and can be ordered separately.

Spare Parts and Accessories

- 5065-4413 – Electrode cartridge
- 5065-4492 – Pressure cartridge
- 5065-4478 – Pressure Adapter Kit
  Contains 5 plastic adapters and 1 mounting ring for use with the pressure cartridge
- G2938-68100 – Test Chip Kit for Electrophoretic Assays
  Comprises 1 Autofocus, 1 Electrode/Diode, and 5 Leak Current Clips
- G2938-68200 – Test Chip Kit for Flow Cytometric Assays
  Comprises 1 Cell Autofocus Chip
- G2938-81605 – RS 232 cable
  Communication cable PC – Agilent 2100 Bioanalyzer
- 2110-0007 – Fuse
- 5042-1398 – Adjustable Clip for use as spare part for the chip priming station
- 5065-4401 – Chip Priming Station including gasket kit and adjustable clip
- G2938-68716 – Gasket Kit
  Includes spare parts for the chip priming station: 1 plastic adapter, 1 ring and 10 gaskets
- 5065-4428 – IKA Vortexer (115V)
  Must be ordered at IKA (http://www.ika.de)
- 5065-4429 – IKA Vortexer (230V)
  Must be ordered at IKA (http://www.ika.de)

- 5065-9966 – Vortex Mixer Adapter for IKA vortexer
- 5065-9951 – Electrode Cleaner Box

  Contains 7 electrode cleaners
- G2946-60002 – Agilent 2100 bioanalyzer How to Use CD-ROM

  Contains videos showing the chip preparation for all assays and the hardware maintenance

Glossary

This glossary explains terms in context with flow cytometry, electrophoresis, and software or hardware of the Agilent 2100 bioanalyzer.

A

Area Threshold

The Area Threshold setpoint determines the minimum amount of peak area that must be detected before a peak is recognized.

Assay

An assay is a solution with defined chip, chemicals, instrument methods, data analysis, data output settings and data display settings.

ASY file

In *Bio Sizing* electrophoretic assays were stored as .asy files. 2100 expert can import .asy files. See also XSY file.

Audit Trail

Audit trails are available in the 2100 expert software only with the security pack installed. They are used to record the activities of the logged-in users and cannot be modified. The audit trails as well as log books are subject to data protection. Only authorized users are allowed to inspect them. They are saved with the data files or into an audit file repository, which is automatically archived.

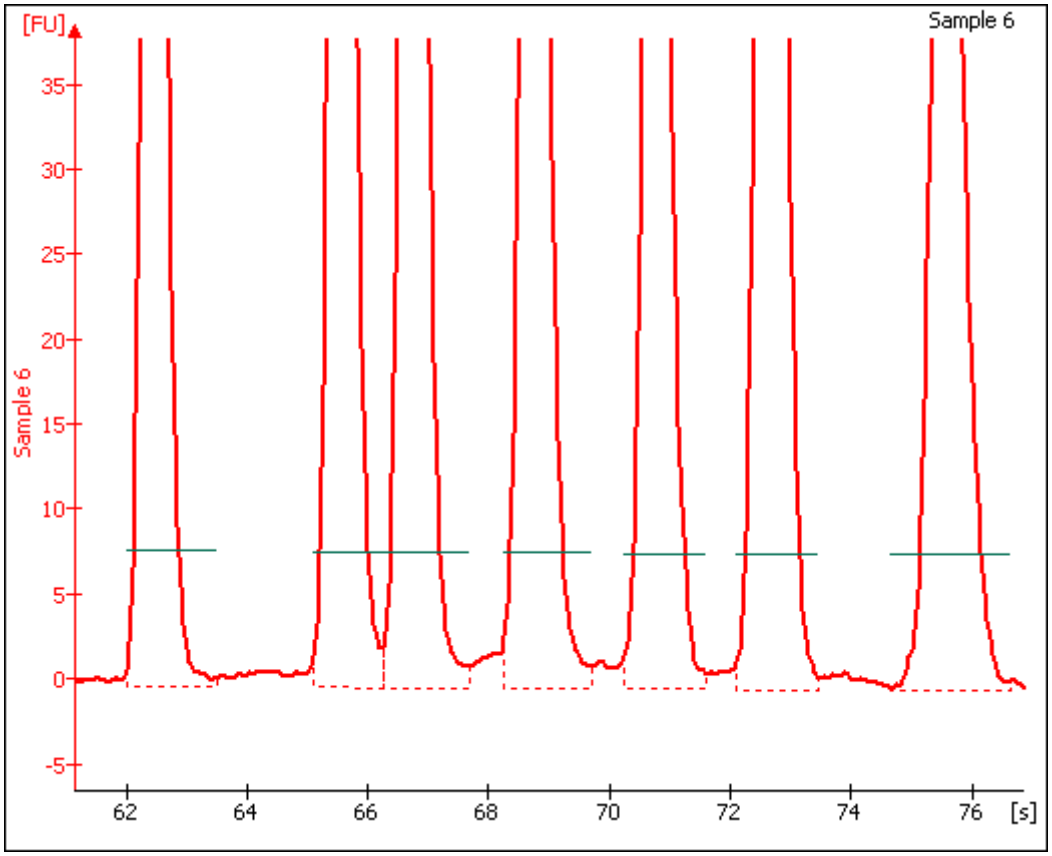
B

Baseline

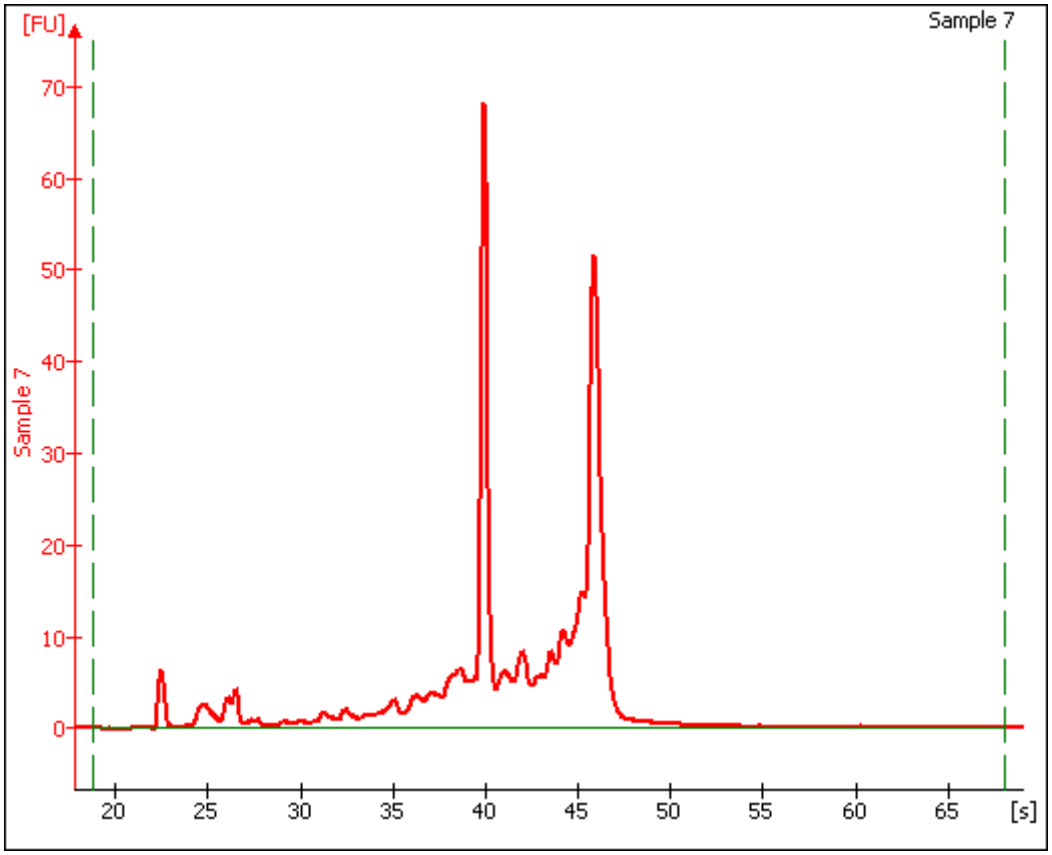
A baseline is established just after the First Peak Time setpoint. After the overall baseline is established, a local baseline is calculated for each peak to compensate for baseline drift.

For isolated peaks, the local peak baseline is simply a straight line connecting the Start Point of the peak with the End Point. For peaks that are very close together, an average baseline is used when the value between the peaks does not drop to the actual baseline.

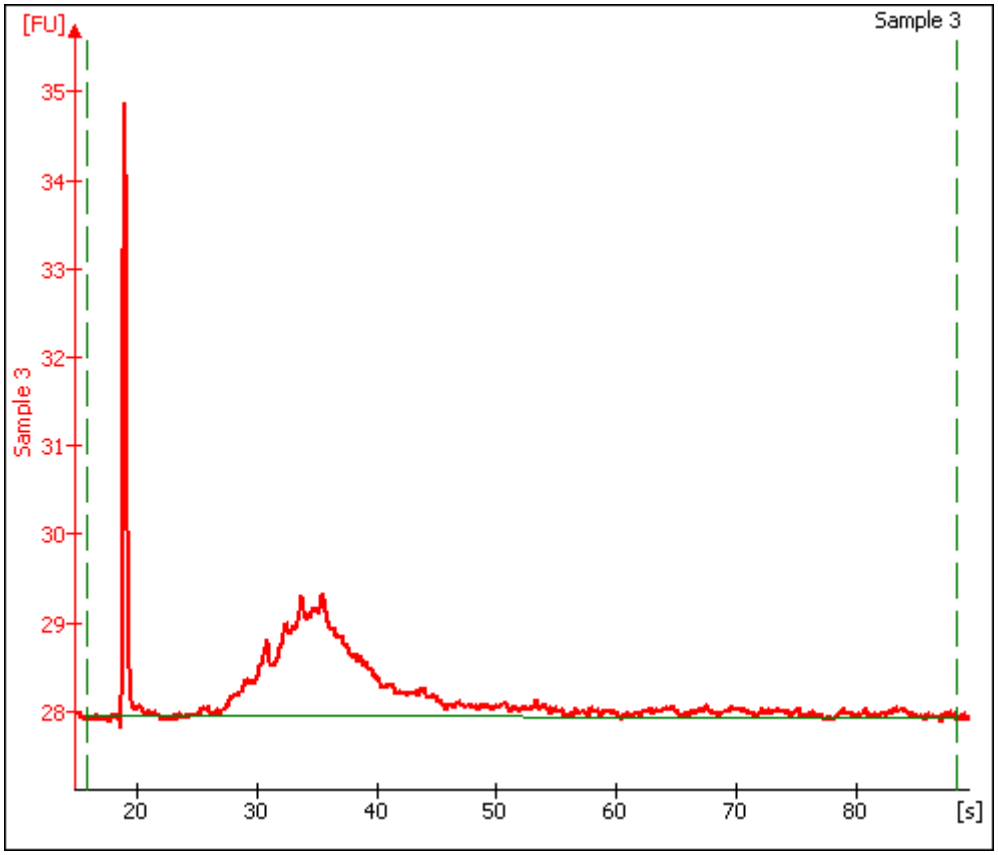
The figure below shows baselines established for DNA assay peaks. Peaks for DNA and protein assays are determined on a peak-by-peak basis (the overall baseline is shown).



The figure below shows baselines established for Total RNA assay fragments. Total RNA fragments are determined on a peak-by-peak basis and an overall baseline is shown from the start to end time.



The figure below shows baselines established for an mRNA assay. mRNA fragments are determined on a peak-by-peak basis and an overall baseline is shown from the start to end time.



NOTE

With RNA assays, you can move the lines marking the start and end points for data analysis (shown by the long-dashed vertical green lines) which will adjust the entire baseline for calculation of the area of the total sample.

Baseline Plateau

This setpoint (found in the setpoint explorer) rejects brief, low slope areas such as at peaks and between non-baseline-resolved peaks. The signal is recognized to be at baseline whenever the slope of the data is less than the Slope Threshold setpoint (either positive or negative) for longer than the time set for the Baseline Plateau.

BMP file

BMP is the standard Windows image format. The BMP format supports RGB, indexed-color, grayscale, and bitmap color modes.

Bubble

If the tip of a pipette is not positioned all the way to the bottom of a well, bubbles can result (and sometimes bubbles happen even when you are very careful). The vortexing step that occurs after samples are loaded into the chip is designed to rid the wells of bubbles and is usually very effective.

If a large bubble is seen at the bottom of a well, remove the sample from the well, pipette it back in, and continue with the loading procedure.

C

CAD file

In *Cell Fluorescence* flow cytometric chip runs were stored as .cad files. 2100 expert can import .cad files. See also XAD file.

Center Point

After locating a start point, the peak find algorithm looks for the first negative slope value and saves the previous point as the center. If the value of the center point is less than the Minimum Peak Height, the algorithm starts looking for a new peak.

CLD file

In *Bio Sizing* electrophoretic chip runs were stored as .cld files. 2100 expert can import .cld files. See also XAD file.

COM Port

See Serial port.

CSV file

Comma-separated variable file. The simplest form of file for holding tabular data. Data is listed in columns in a text file, each value being separated by a comma. Each new line represents a new set of data. Import and export with Microsoft Excel is possible.

CSY file

In *Cell Fluorescence* flow cytometric assays were stored as .csy files. 2100 expert can import .csy files. See also XSY file.

D

Data Filtering

The first step *2100 expert* takes in analyzing raw data is to apply data filtering. Data filtering is done by means of a polynomial "filter" that is applied to the raw data. The setting for the Polynomial Order in the setpoint explorer determines the amount of data to be applied: the smaller the number, the more data that is applied and the more filtering that takes place.

Data Points

Data points are 0.05 seconds apart.

Show Data Points is an option that enables the display of the data points used to generate the graph.

E

Electrode Cleaner

An electrode cleaner should be used to clean the electrodes after each run is complete. The cleaning procedure is slightly different depending upon the type of assay that was just performed (DNA or RNA).

The electrode cleaner looks like a chip except that it is clear. With RNA assays you must use two different electrode cleaners: one for general cleaning using RNAse-free water and another for decontamination using RNAseZAP. It is recommended to use a permanent marker to label the electrode cleaners so as not to mix them up.

Electrokinetic forces

Electrokinetic forces are used to move, switch and separate the samples. Active control over voltage gradients directs the movement of materials using the phenomenon of electrophoretic flow.

Electroosmotic Flow

A phenomenon that results from an electrical double layer formed by ions in the fluid and surface electrical charges immobilized on the capillary walls. When an electric field is applied, the bulk solution moves towards one of the electrodes. This phenomenon can be used to move fluids through microfabricated channels.

Electrophoresis

A standard technique of separating molecules on the basis of their mobility (charge-to-mass ratios). An electrical potential is applied across a capillary containing a sample in a fluid medium. Positive molecules migrate towards the cathode and negative molecules migrate towards the anode at different speeds, depending on their electrophoretic mobility.

Electrophoretic flow

A macroscopic phenomenon that results from an electrical double layer formed by ions in the fluid and surface electrical charges immobilized on the capillary walls. When an electric field is applied, the bulk solution moves towards one of the electrodes (cathode). Electrodes sit in the reservoirs that connect to the ends of the various channels. Electrode potentials are applied to the various reservoirs in a time-dependent fashion to move the fluid in the required direction. The gel-filled channels of the LabChip devices do not exhibit a measurable flow because of dynamic channel coating and viscosity of the polymer matrix.

End Point

The peak find algorithm looks for a leveling off when the value of the slope is less than the value set for the slope threshold. This is considered to be the end point of the peak.

With RNA assays, individual peak end times can be moved manually by dragging the diamond-shaped end points shown in the single-well display.

End Time

This setpoint determines the time after the start of a run before which the last peak or fragment will be located (any peaks appearing after this time are ignored). In RNA assays, the end time is shown on the single-well display as a long-dashed vertical green line.

With RNA assays, another End Time setpoint is available that controls the end time for an individual peak. Additionally, individual peak end times can be moved manually by dragging the diamond-shaped end points shown in the single view.

F

Filter Width

This setpoint determines the width of the polynomial (in seconds) to be applied to the data for filtering (noise reduction). The default depends on the assay selected. This setting should be less than twice the width of the peaks of interest or the peaks will be distorted. Peaks that are distorted by the filter have positive and negative peaks on both sides. To see an example of such distortion, increase the filter width to 5.

Firmware

The firmware is a program to control the hardware of the Agilent 2100 bioanalyzer. It is downloaded from your computer to the Agilent 2100 bioanalyzer and controls, among others, data transfer or the measurement procedures.

Flow Cytometry

A method to detect cells with certain properties. In a continuous stream, stained cells pass through a light beam. The emitted fluorescence is used for counting and differentiation.

Flow Cytometry Standard—FCS

The FCS file format is the standard format used in flow cytometry to exchange data between several applications.

G

GIF file

Graphics Interchange Format, GIF is a graphics file format that uses a compression scheme originally developed by CompuServe. Because GIF files are compressed, the file can be quickly and easily transmitted over a network. This is why it is the most commonly used graphics format on the World Wide Web.

H

Height Threshold

The Height Threshold setpoint determines whether a peak is kept. It represents the minimal peak height. For each peak, the difference between the start point value and the

center point value (local baseline) must be greater than the *Height Threshold* value.

This setting is chosen in the setpoint explorer.

Histogram

Histograms are bar charts to display, for example, a frequency distribution.

HTML file

HTML (Hyper Text Markup Language) is the authoring language used to create documents on the World Wide Web. HTML defines the page structure, fonts, graphic elements and hypertext links to other documents on the Web.

J

JPG file

Joint Photographic Experts Group Image File. A JPEG file is a compressed raster or bitmapped graphic image. When a JPEG is created, a range of compression qualities may be considered. JPEG compression is a lossy process, which means that you sacrifice quality for file size the more you compress the image (the highest quality images results in the largest file size). Whereas GIF images are limited to 256 colors (8-bit), JPEG images may contain millions of colors (24-bit) as well as additional information including PostScript clipping paths.

L

Lab-on-a-chip

The generic term for a microfluidic product, signifying a chemical process or material movement taking place on a microchip. In contrast to analysis in a standard laboratory that relies on human intervention at several stages to manipulate or observe samples and record results, the self-contained lab-on-a-chip represents an almost hands-free technology.

Lab-on-a-chip technology means downsizing of analytical techniques from lab-scale to chip-scale:

- using techniques like electrophoresis, chromatography, and sieving.
- with fluorescence, absorbance, and MS detection.
- with a higher degree of automation, integrating multiple steps of a complex protocol into a miniaturized system.

Virtually any biochemical testing that can be done in a laboratory can theoretically be done on a chip.

Ladder

Each electrophoretic LabChip Reagent kit contains a ladder. A ladder contains DNA, RNA fragments or proteins of known sizes and concentrations.

A ladder well is located at the bottom right of the chip. The ladder is analyzed first before sample analysis takes place.

The peak sizes and markers defined for the ladder are assigned consecutively, starting with the first peak detected in the ladder. Peaks appearing above the upper marker do not have to be detected. The peak table for the ladder well shows the peak size and concentration.

Lower Marker

An internal standard that is added to a sample in a well to assist in determining size of the sample. The lower marker is the same as the first peak found in the DNA ladder.

M

Method

Methods are available in the 2100 expert software only with the security pack installed. A method is referred to as an electrophoretic or flow cytometric assay with additional information stored to it. This additional information includes instrument information, study information, report settings, and workflow definitions.

Microfluidics

The movement of liquids through micro-fabricated structures by means of electrical fields or pressure/vacuum, holding the promise of greater functionality with significantly improved reliability:

- small glass or plastic devices with micro-channels as experimental platform
- active control of fluids without moving parts on-chip through miniature electrodes or pumps controlled by software scripts
- emulation of conventional liquid pumps, valves, dispensers, reactors, separation systems, etc.

- capability of liquid transfer, separation, dilution, reactions and more

Molarity

$$Molarity = \frac{Concentration * 10^6}{660 * Size} \left[\frac{nmol}{l}\right]$$

where:

Molarity is measured in nanomoles per liter (nmol/l)

Concentration is measured in nanograms per microliter (ng/µL)

Size is measured in base pairs (bp)

$660 \left[\frac{g}{mol * bp}\right]$ is the molecular weight of a single base pair

Miniaturized laboratories on a microchip

Expression used to describe lab-on-a-chip technology.

Molecular separation techniques

Processes such as gel electrophoresis, liquid chromatography and capillary electrophoresis that can separate bimolecular organic substances from other compounds.

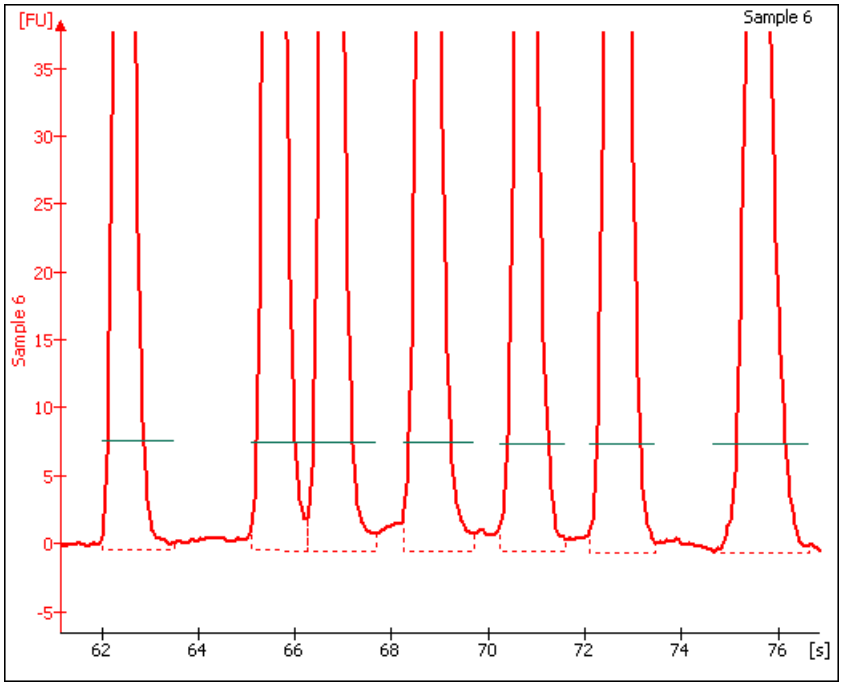
P

PDF file

PDF (Portable Document Format) is a file format created by Adobe Systems Incorporated that preserves all of the fonts, formatting, colors, and graphics of any source document, regardless of the software and computer platform used to create it.

Peak Baseline

A local peak baseline is calculated for each peak. For isolated peaks, the local peak baseline is simply a straight line connecting the start point with the end point. For peaks that are very close together, an average baseline is used when the value between the peaks does not drop to the actual baseline.



Peak Filter Width

The Peak Filter Width setpoint determines the minimum amount of time that must elapse before a peak is recognized.

Peak Height

The value at the center point of the peak minus the local baseline start value.

Point-to-Point Fit

This curve fit is composed of line segments between each pair of data points that are used to interpolate data between those points.

Polynomial Filter

The first step *2100 expert* takes in analyzing the raw data is to apply data filtering. Data filtering is done by means of a polynomial "filter" that is applied to the raw data.

Priming Station

Consists of a chip holder that has a syringe mounted on the lid that seals over the chip. The syringe is used to force the buffer solution loaded into the well marked "G" with a circle around it into all the passageways inside the chip prior to running it in the bioanalyzer.

S

Serial port

The serial ports (COM ports) are used to connect your computer with the Agilent 2100 bioanalyzer. The number of available ports depends on the computer you use.

Signature

Signatures are available in the 2100 expert software only with the security pack installed. All activities on data such as creating, modifying, and deleting data must be confirmed by the user with an electronic signature (user name and password). By requesting this signature it is ensured that only authorized users can create, modify, and delete data.

Slope Threshold

The Slope Threshold setpoint determines the difference in the slope that must occur in order for a peak to begin. The inverse of this value is used to determine the peak end.

Standard Curve

The standard curve is obtained by plotting the size of the ladder peaks vs. time using a point-to-point fit. For each sample peak, the center time is interpolated from the Standard Curve to determine the peak size in base pairs.

Start Point

The peak find algorithm walks the data from time zero looking for a slope greater than the Slope Threshold. This is considered to be the start point of a peak.

With RNA assays, individual peak start times can be moved manually by dragging the diamond-shaped start points shown in the single view.

Start Time

This setting determines the time after which the first peak or fragment will be located (any peaks appearing before this time are ignored). In RNA and Protein assays, the start time is shown on the single view display as a long-dashed vertical green line (note that this is true for protein assays when analysis is on; the start time is shown as a solid green line when analysis is off for protein assays).

With RNA assays, another start time setting is available that determines the start time for an individual peak. With RNA assays, individual peak start times can be moved manually by dragging the diamond-shaped start points shown in the single view.

T

Tool Tip

A small box containing text that describes the item indicated by the mouse pointer. To view a Tool Tip, position the mouse pointer over an object on the screen. Leave the mouse stationary for a moment and a Tool Tip (if one exists for that item) will appear.

TIF file

A file extension indicating one of a set of popular bitmap graphics formats. Tiffs are commonly used in DTP work because of their support for color specification.

U

Upper Marker

An internal standard that is added to a DNA or Protein sample in a well to assist in determining size and concentration of the sample. The upper marker is the same as the last peak found in the sizing ladder.

W

WAV file

A type of computer file used to store a sound digitally.

Workflow

The workflow defines the order of steps that need to be taken for a measurement to ensure data validity and data reliability. This includes steps such as the execution of methods, result reviews, and the final approval.

The workflow definition is part of the methods and is available in the 2100 expert software only with the security pack installed.

WMF file

Windows Metafile. Windows metafile documents can contain any mix of vector and raster (or bitmapped) information to describe the contents of an image. WMF graphics are generally used on the Windows platform as a standard format for clip art and other graphically rich information such as charts.

X

XAD file

2100 expert chip data file. The files contain raw data, assay information, data analysis setpoints, information on chip, samples and study, and the run log information.

XAC file

2100 expert comparison file.

XLS file

Microsoft Excel spreadsheet file.

XML file

Extensible Markup Language files. XML is the Extensible Markup Language, a system for defining specialized markup languages that are used to transmit formatted data. XML is conceptually related to HTML, but XML is not itself a markup language. Rather it is a metalanguage, a language used to create other specialized languages.

2100 expert uses the XML format to:

- export chip data
- save and load result flagging rules.

XSY file

2100 expert assay file. The files contain the assay properties, data acquisition settings, and information on chip, samples, and study.

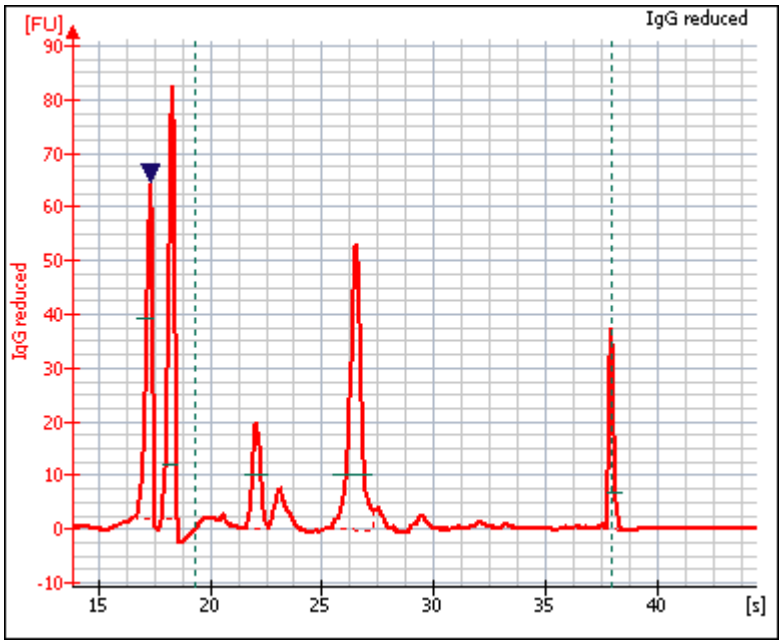
XVD file

2100 expert verification result file. The files contain results of verification tests regarding the bioanalyzer hardware and software. xvd. files are stored in the "..\validation" subfolder of the 2100 expert installation directory. For each verification run, an .xvd file is generated.

Date and time of the verification run are included in the file name. Example: "Verification_23-05-2005_10-28-40".

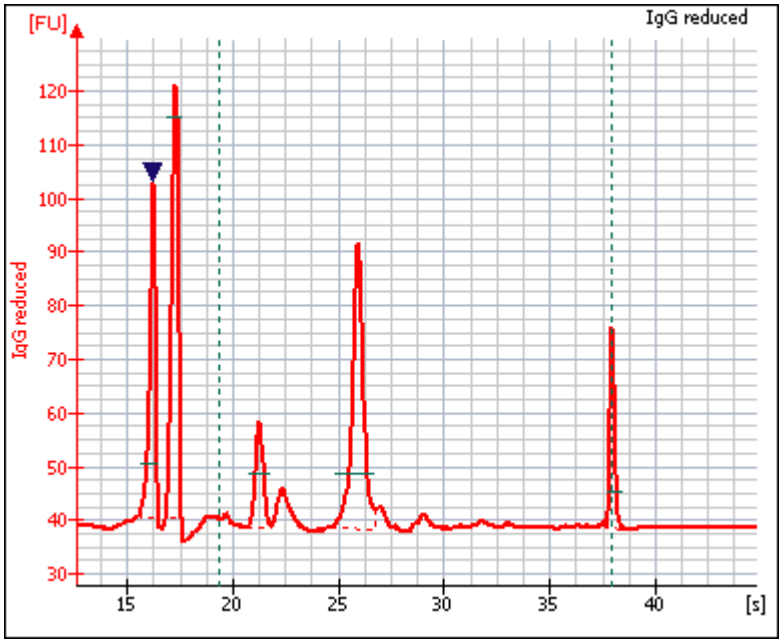
Zero Baseline

All electropherograms produced with the bioanalyzer show some amount of background fluorescence. By default, the *2100 expert* software enables the zero baseline function. Enabling this setting offsets the graphs shown for the individual wells but does not affect analysis. The mean of 100 points before the baseline time (derived when calculating well noise) is used as the zero baseline value.



Zero Baseline

To remove the zeroing, disable the *Zero Baseline* box in the setpoint explorer (baseline calculation under *Global* and *Advanced* setting).



None-Zero Baseline

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